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# PROCEEDINGS OF THE SIXTH INTERNATIONAL CONGRESSES ON TROPICAL MEDICINE AND MALARIA

INSTITUTO DE MEDICINA TROPICAL

# PROCEEDINGS OF THE SIXTH INTERNATIONAL CONGRESSES ON TROPICAL MEDICINE AND MALARIA

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DIVISION A

# TROPICAL MEDICINE

*(Continued)*

SECTION 2

# PROTOZOAL INFECTIONS

SUBSECTION 1

## TRYPANOSOMIASIS

1ST SESSION: **African trypanosomiasis**

*Tuesday, September 9th — 9:00 to 10:30 a. m. Room D*

The meeting was held under the chairmanship of Dr. P. Richet and Dr. M. A. de Andrade Silva was the Rapporteur. The Secretary was Dr. Augusto R. Pinto.

The following papers were presented and discussed.

## SOME ASPECTS OF TRYPANOSOMIASIS IN AFRICA

M. A. DE ANDRADE SILVA

Director, Missão de Combate às Tripanossomíases, Moçambique

The geographical distribution of the tsetse fly, which is neither regular nor homogenic, extends between the paralels 14° North and 29° South on the African continent. It is calculated that an area over 11,000,000 km² is involved.

Including the recent identification of *G. vanhoofi* Henrard, 1952, and *G. nashi* Potts, 1955, and taking into account that *G. fuscipes* is considered a distinct species from *G. palpalis* (de Barros Machado, 1954), today the total number of species of glossina is 23.

The existence of the tsetse fly in Africa has been and continues to be a cause for the slow development and progress in enormous areas, as some species transmit sleeping sickness and all species, as we believe, can transmit animal trypanosomiasis. Consequently, the presence of tsetse fly is disastrous: apart from carrying diseases it causes poverty.

Epidemics of sleeping sickness provoked depopulating of some areas: we only have to mention the catastrophe at the beginning of this century along the shores of Lake Victoria. Even today this disease causes loss of human life and can still present itself in epidemic form.

Depending from the species and local circumstances and conditions, the tsetse fly can cause the absence of domestic animals or, where cattle does exist, the fly greatly enhances the difficulty of breeding. Today animal trypanosomiasis has perhaps a greater importance than sleeping sickness, as it is distributed over a much larger area. The area of nagana is in fact larger than the actual tsetse fly belts.

Animal trypanosomiasis interferes badly with the life of the population: it causes malnutrition because of the lack of animal proteins, it hinders their economy and can even bring about social trouble.

The lack of cattle is also a reason for the backward shifting-cultivation of the Africans.

If Africans had cattle and knew how to use manure and employed animals to till the soil, their farms would acquire a greater stability and would pass from father to son, thus creating true rural properties, which now seldom happens. This would result in settlement of the Africans on the land and consequently produce better crops, improve cattle breeding, and general prosperity.

In East Africa animal trypanosomiasis is certainly of more importance than sleeping sickness.

It is the aim of this paper to show some aspects of the problems brought about by the tsetse fly in the face of scientific knowledge and work done previously and still in progress. The short space of time confines me to essential questions and does not allow to go into matters also important.

## SLEEPING SICKNESS

As a result of the enormous progress in the chemotherapy, today it is much easier to control sleeping sickness.

In the last 20 years or so the geographical distribution of sleeping sickness has changed little.

Where chemoprophylaxis was applied a dramatic fall of the incidence of Gambian trypanosomiasis was noted. There were even some isolated focci which are now completely free of the disease.

The Rhodesian trypanosomiasis in East Africa has kept its characteristics of distribution: in widely scattered areas. As a rule, the disease is latent, with a very low incidence of less than 1 per thousand persons examined. 1 to 2 percent incidences are rare, only during epidemic outbreaks.

Apart from the territories where *T. rhodesiense* sleeping sickness was known long ago. it appeared for the first time in Urundi in 1954 (Marneffe, 1955) in the epidemic form, with an incidence of 1.35 percent.

Great lacuna exist in the knowledge of the epidemiology of sleeping sickness. We do not know why the disease is always present in some areas and not in others, although the same vectors and similar conditions prevail.

Also we do not know exactly the reasons for the epidemic outbreaks.

More research work is needed on the epidemiology studying the factors which further the spread of the disease and those which restrain it. Some of these factors are to be looked for in the animals on which the fly feeds, other factors must be sought in the biology of the trypanosomes, and in the tsetse fly itself.

By this study we may well discover vulnerable spots in the transmission of the disease so as to be applied in the control.

Morris (1951) studied in Ghana the epidemiology of the Gambian trypanosomiasis according to ecological factors. The tsetse fly and the population played important roles in the occurrence of epidemics: the fly producing high rates of infection and the population by spreading the disease from the initial focus to other areas. The disease concentrated itself in the more populated areas along the rivers and from there it was spread by people travelling on the roads and other lines of communication.

In Mozambique we have recorded some epidemiological aspects of *T. rhodesiense* sleeping sickness (de Andrade Silva, 1952). In this

territory the endemic areas closely accompany the permanent water courses, a fact related to the location of the villages which is forced by need of water.

The residual areas of *G. morsitans* lie usually near the water courses, on account of the more suitable vegetation, in the dry season. Game in its turn may gather during the same season near the villages to drink in the pools formed by the river or in the nearly lagoon, the only water available over a large area.

In these circumstances man, game and tsetse fly are forced to a closer contact by a common need for food.

We established that over a great number of years the location of the endemic areas in Mozambique suffered little alteration.

Large areas of *G. morsitans* sometimes overlapping *G. pallidipes* continue to be free of sleeping sickness. In extensive *G. morsitans* fly-belts we come across endemic areas and areas free of the disease.

In three areas formerly free of sleeping sickness occurred epidemic outbreaks, which always could be traced back to patients coming from infected areas, the same as Fairbairn (1944) pointed out in Tanganyika.

Trypanosomes must have a capital importance in the epidemiology of sleeping sickness. Unfortunately in this field too little is actually known. Neither the causes which influence the transmissibility of trypanosomes, by the tsetse fly, nor the factors which rule the infectivity to man are exactly known.

The differenciation of the *T. brucei, T. gambiense,* and *T. rhodesiense,* whose morphology is identical, in distinct species, continues to be discussed.

The arguments whether *T. brucei* and *T. rhodesiense* are the same species but with different infectivity and pathogenic action continue.

The relations between the above mentioned trypanosomes are still to be solved.

*T. brucei* does not infect man, and the distinction between this trypanosome and *T. rhodesiense* can only be made by the infectivity test in man. Both trypanosomes are very virulent to small laboratory animals. They don't show morphological differences, and during infections in laboratory animals postero-nuclear forms are frequently found. Both are resistant to tryparsamide.

*T. gambiense* and *T. rhodesiense* infect man. The small laboratory animals are less susceptible to *T. gambiense*. Tryparsamide is usually active against the latter trypanosome.

*T. gambiense* infections frequently take a slow course, but strains encountered in epidemic areas proved very virulent, in guinea pigs these strains caused infections with similar course of those of *T. rhodesiense* and even postero-nuclear forms can be found in the blood.

In man these virulent strains give high parasitisme in the blood in contrast with the absence of gland enlargement, and the course of the disease is rapid, with cerebrospinal fluid changes within two or three months as does *T. rhodesiense*. These *T. gambiense* strains are resistant to tryparsamide.

*T. rhodesiense* is associated with flies of group *morsitans* and *T. gambiense* with species of group *palpalis*.

Willet (1956), discussing the origin of *T. rhodesiense*, thinks it a virulent form of *T. gambiense* changed by cyclical transmission in flies of the *morsitans* group, namely *G. pallidipes*. He alternatively suggests that the most virulent strains of *T. gambiense* would be transmissible by tsetse flies of the *morsitans* group and so converted into *T. rhodesiense*.

We have not observed a constant association between the appearance of Rhodesian trypanosomiasis and the presence of *G. pallidipes*. For instance, in Mozambique the first cases of *T. rhodesiense* sleeping sickness were recorded between 1909 and 1912 in the Makanga area, but neither at that time nor today *G. pallidipes* was found there. We have to admit however that in some areas *G. pallidipes* coexists with *G. morsitans* and the first species is bound to play a part in the transmission of this disease.

We deem it of importance to investigate the importance of *G. pallidipes* in relation to *G. morsitans* as a vector and whether the virulence of the trypanosome is augmented through the former species.

The importance of game as reservoir of trypanosomes up to now has not been properly valued.

Rare species of mammals of the wild fauna are immune to infections by pathogenic trypanosomes, including trypanosomes of

*brucei* group, but circumstances make it difficult to fix the exact percentage of infected animals.

A hematological survey of game to find trypanosomes produces uncertain results even if it includes a large number of animals. The main difficulties of a survey consist in defficiency of blood smears and the cryptic character of the trypanosome infections in game animals. Besides, it is impossible to distinguish between the trypanosomes of *brucei* group by their morphology.

It is not enough only to search for trypanosomes in the blood. It is also necessary that blood of wild animals should be injected in small laboratory animals in order to study the trypanosomes where present.

Fairbairn (1955) gave a list of species susceptible to *T. rhodesiense* infection. As far as *T. gambiense* is concerned, the study to determine the respective reservoirs has been less profound. According to van Hoof (1948), *T. gambiense* provokes chronic infections without clinical symptoms in pigs and other domestic animals, but the parasite is easily cyclically transmitted from animal to animal and even to man.

The transmission of sleeping sickness in its simplest way is from man to fly, and back to man.

In certain circumstances we have to admit to a possible chain man → fly → game →fly → man or game → fly → man.

The percentage of infective fly is very low. In Mozambique we have found an infection rate of the salivary glands (*brucei* group) less than 0.3 percent by dissecting more than 35,000 *G. morsitans* caught in infected areas. It is not possible to distinguish in the tsetse fly between *T. brucei* and *T. rhodesiense*.

Besides, humans do not easily contract sleeping sickness. The receptivity of man depends largely on his resistance, and on the dose of metacyclic trypanosomes injected by the tsetse flies.

The absence of sleeping sickness in some *G. morsitans* areas is one of the major misteries of its epidemiology. How can we explain the lack of cases in these areas, some of which are situated near the endemic areas?

Does the climate affect the cyclical transmission of trypanosomes? Fairbairn (1948) has observed that the incidence of sleeping sickness varies with the monthly mean maximum temperature. Burtt (1946)

has observed in the laboratory that tsetse flies emerged from pupae, kept at temperatures of 30°C, acquired *T. rhodesiense* infections at a three times higher rate than those kept at lower temperatures.

Vaucel (1949) suggested that certain climatic conditions would be particularly favourable to the receptivity and virulence of the trypanosomes in the tsetse fly.

Also on this subject more knowledge would be welcome.

Later in this Congress Drs. Fairbairn and Willet will read very interesting papers on protozoological questions.

Passing on to the subject of chemotherapy, the most valuable drugs which are in use today are no doubt: Bayer 205 (suramin, antrypol, moranyl), the diamidines, especially the diaminodiphenoxypentane salts (pentamidine, lomidine) and the pentavalent arsenical Mel B Friedheim (arsobal).

Arsobal was an enormous step forward in the therapeuthics of sleeping sickness because it enables us to affect a high percentage of cures in nervous cases, even in *T. rhodesiense* infections, which with tryparsamide treatment would be fatal.

Today arsobal is the most extensively used drug.

The International Scientific Committee for Trypanosomiasis Research (I.S.C.T.R.) did much to encourage the use of arsobal by discussing the experience obtained in different territories and recommending general schemes of treatment.

The same Committee established norms for the use of other drugs.

For the choice of treatment it is essencial to study and classify the clinical cases.

Bayer 205, pentamidine and arsobal are powerful trypanocides, each in different degrees.

Arsobal is very active both in the hemolynphatic and nervous stages, but Bayer 205 and pentamidine are only effective in the hemolynphatic stage.

Tryparsamide, although a weak trypanocide, acts favourably in the nervous stage, specially of *T. gambiense* sleeping sickness, with the exception of resistant cases, and is still in use.

As we have mentioned, mass prophylaxis with pentamidine was an enormous success in several West African territories: the incidence

of the disease diminished considerably. In French Equatorial Africa, French West Africa, Angola, Belgian Congo and other territories, pentamidine injected every 6 months gave unprecedent results, the index of circulating virus came down to 0.5, 0.1 percent and even caused the complete disappearance of sleeping sickness in some areas.

The new cases became rare, but from this excellent position acquired at such great effort to the complete elimination of the disease is still a great distance.

Van Hoof (1948), writing about residual trypanosomiasis, already foresaw this. He pointed out the necessity to consider the existence of other trypanosomes reservoirs besides man, the different strains of parasites as for their virulence, transmissibility and infectivity in different areas, and still the difficulty to discover the trypanosomes in patients.

The most important factor for the chemoprophylaxis is the control of the population. All of us with experience in fighting diseases in Africa know that many don't answer the call. All depends on their cooperation, if not many stay behind exposed to infection, and possible patients amongst these act as reservoirs of trypanosomes.

Neujean (1954) points out that pentamidine can produce parasitological camouflage. Difficulties of diagnosis increased, to find trypanosomes is sometimes extremely difficult, forcing us to use all methods of diagnosis.

Dr. Reimão Pinto, in his paper, will mention both the usual and the more complicated methods of diagnosis.

The complement-fixation test would be of great help in the above mentioned cases, but unfortunately this test is not specific. When positive, it does not allow us to ascertain exactly the diagnosis of trypanosomiasis.

As long as the vector insect is in contact with reservoirs of trypanosomes, the possibility of spreading the disease remains, in proportion to the transmissibility of various strains of trypanosomes.

The control of *T. gambiense* sleeping sickness should continue under the close survey of the sanitary authorities, if not, the position will aggravate.

Prof. Neujean will give us later on a very interesting talk on treatment and chemoprophylaxis of Gambian trypanosomiasis.

Treatment of *T. rhodesiense* sleeping sickness is conducted more or less on the same lines as *T. gambiense*.

Arsobal is the most active drug, giving a large percentage of cures both in haemolynphatic and nervous stages. However we prefer antrypol and pentamidine in early cases because arsobal, being very toxic, is difficult to administer.

In these early cases, classified through careful examination of the cerebrospinal fluid, both antrypol and pentamidine give good results. But when we find even slight changes of the C.S.F., we have to use arsobal.

This point is of capital importance. We consider as normal a fluid with a maximum protein contents of 0.25 g $^{0}/_{00}$ and with leukocitosis less than 3 cells per cubic millimeter.

Anything above these values means that the disease has passed the haemolynphatic stage. Yet it is possible to find a normal fluid in cases having already meningeal reaction. In these cases antrypol and pentamidine are not active.

Antrypol is given intravenously and usually in a course of 5 injections: the 1st, 3rd, 7th, 14th and 21st days.

Pentamidine is given intramuscularly at the dosage of 4 mg/kg body weight during 10 consecutive days.

Results obtained with both drugs are similar, with perhaps antrypol slightly the better of the two.

Both with antrypol and pentamidine, a small percentage of relapses is observed. These cases have to be treated afterwards with arsobal.

At the 6th meeting of the I.S.C.T.R., held in Salisbury, we presented a paper on the value of the drugs used in the treatment of Rhodesian sleeping sickness (de Andrade Silva, 1956). In this paper we have recorded the results obtained in numerous cases treated and followed up after treatment.

In the early cases no doubt it is arsobal which gives the best results. Until now we have treated more than 50 of these cases. No relapses were observed either parasitological or of the C.S.F. and 79 percent of the treated cases were considered to be cured two years after treatment. Some cases only received a single injection of 3.6 mg/kg body weight.

We have treated recently 39 early cases with antrypol + pentamidine as follows:

1st day: 1 g of antrypol in the vein + 4 mg/kg pentamidine intramuscularly.
3rd and 7th days: same dosage of both drugs.
8th, 9th, 10th, 11th and 12th days: 4 mg/kg of pentamidine intramuscularly.

Good results were obtained.

Now in Mozambique we use arsobal practically in all nervous cases, either alone or with tryparsamide.

In 1951 we started using arsobal and from then onwards we treated hundreds of cases with this drug (de Andrade Silva *et al.*, 1953, 1954, 1956).

Tryparsamide as a basic drug was put aside and only we use it in cases with bad condition where we are afraid to administer arsobal. Treatment of such cases begins with antrypol or pentamidine + tryparsamide. As a rule we give 3 injections of antrypol or pentamidine and 4 injections of tryparsamide: 4 cg/kg body weight. After an interval of two or three weeks arsobal treatment is started.

Tryparsamide improves rapidly the general condition of the patients.

The serious accident of toxic encephalopathy is more frequent in patients in bad condition.

Previous administration of tryparsamide avoids the massive destruction of trypanosomes caused by arsobal when given at the start. The sudden hypertermia following the first injection of arsobal may be the signal of this destruction.

The total dose of arsobal to be given depends on the degree of C.S.F. changes and the clinical symptoms.

As a rule we give a dose of 3,6 mg/kg.

Treatment by arsobal consists of one, two or three courses of 3 or 4 injections at consecutive days.

The second and third courses, where necessary, are repeated at an interval of one or two weeks.

The injection must be given carefully, and taking precautions to avoid accidents. Patients must rest on the days of injection and

are kept on a diet. Alcohol is strictly prohibited and patients should be kept under careful observation.

Mild toxic accidents happen which disappear spontaneously.

Sometimes, however, serious accidents occur, the most feared of which is the arsenical encephalopathy. It begins with tremor, immediately followed by convulsions, fever and afterwards coma. It is necessary to act quickly: intramuscular injections of B.A.L. every 3 hours.

Even so, very few cases of encephalopathy are saved.

The statistics we gave in 1956 (339 cases) showed a mortality rate, by encephalopathy, of 6.9 percent. But from that date onwards the percentage of death through this terrible accident came down to 4.6 percent, maybe because we no longer use arsobal at the start of treatment when patients are in bad condition.

We insist on this point as we have observed that such patients do not very well support this drug. Marneffe (1955) and Apted (1957) made the same observation in patients treated in Urundi and Tanganyika.

The posology of arsobal is of very great importance, as we have to consider at the same time its action and the prevention of the toxic accidents. We use the dose of 3.6 mg/kg recommended by Friedheim because it has been reported that arsobal in smaller dose is less effective.

The percentage of relapses we have observed is smaller than that reported by Apted (1957), but it has to be mentioned that this author has used different schemes of treatment and smaller doses of arsobal (1.8 mg/kg in some cases).

Chemoprophylaxis has not been used as widely in *T. rhodesiense* sleeping sickness as in the Gambian trypanosomiasis.

Bayer 205 is still used in the individual prevention of the disease, its period of protection being estimated to last from two to three months. Pentamidine is also given in Mozambique and other territories to persons more exposed to infection.

Pentamidine was used in mass prophylaxis in Mozambique, to check an epidemic outbreak (de Andrade Silva and Caseiro, 1956). The drug was given every 6 months, for two years, and its dose varied between 4 to 5 mg/kg, but the majority of people received the bigger dose.

The first injection of pentamidine was given to 11,869 people, but many did not turn up for the subsequent ones. 5,077 people were kept as controls, amongst whom 47 new cases were diagnosed over the period of two years. Amongst the 1,869 injected, only 6 new cases were diagnosed in the same period, although many only received the first injection.

Of these 6 cases, 3 had been injected less than 6 months before and we have assumed that pentamidine failed in these cases.

The incidence of the disease came down from 104 cases, in the year preceding the prophylactic campaign, to 17 cases, the year following the end of the compaign. Pentamidine proved itself a useful prophylactic to fight the mentioned epidemic outbreak.

Marneffe (1955) also used pentamidine and moranyl in mass prophylaxis to check an epidemic outbreak of Rhodesian trypanosomiasis in Urundi.

## ANIMAL TRYPANOSOMIASIS

The problem of animal trypanosomiasis, a group of diseases caused by different trypanosomes, is very complex. We have only to consider its vast area of distribution, differences in susceptibility and tolerance of the various species towards the parasites, and the altering effects of the tripanocides in relation to the etiology of the infections.

In this paper we will only mention some of the matters related to animal trypanosomiasis transmitted by the tsetse fly.

The most important trypanosomes are *T. congolense, T. vivax, T. brucei* and *T. simiae.*

The collective term of nagana means especially the infections of *T. congolense, T. vivax* and *T. brucei* and also the mixed infections.

As all domestic mammals are susceptible to *T. congolense* infection, this makes it by far the most important. Bovines are very susceptible and the course of infection depends on the virulence of the strains and the resistance of the animals.

The disease can take acute, subacute or mild forms, the first two giving high rate of mortality.

In horses, donkeys, sheep and goats *T. congolense* infections are usually less severe than in cattle.

*T. vivax* infects, as a rule, cattle, horses, sheep and goats. Its virulence greatly varies from area to area. In cattle *T. vivax* infections can be mild, the animals recovering spontaneously, or severe, provoking high death rate.

*T. brucei* infections are rare in bovines. More susceptible are the horses, donkeys, dogs, goats and pigs. In cattle and pigs this disease is usually benigne.

*T. simiae* infections are very acute and fatal in pigs. In much lesser degree, sheep, goats and even cattle are also susceptible.

All the above mentioned trypanosomes are transmitted by the tsetse fly and this is nature's way of spreading animal trypanosomiasis.

Mechanical transmission by insects of *Tabanus, Haematopota, Stomoxys* and other genera is possible when the introduction of infected animals into a herd occurs. In this event mass treatment by trypanocides usually elliminates the disease.

If in a certain area animal trypanosomiasis is constantly present, it becomes necessary to search for the tsetse fly. Hornby (1952) cites Zanzibar and Inhambane in Mozambique as examples of regions where for a long time mechanical transmission was held responsible for animal trypanosomiasis, that is before *G. austeni* was found.

In other areas in Mozambique where nagana was atributed to mechanical transmission *G. austeni* was also discovered.

The diagnosis of animal trypanosomiasis is not always easy. Animals may suffer from the disease and show all the characteristic symptoms and yet no trypanosomes are found in the blood. This is a common occurrence in *T. vivax, T. congolense* and *T. brucei* infections and could be explained by the usual course of these infections, although it is more frequent with *T. vivax.*

This also happens in reverse: we come across animals in good condition with no symptoms of nagana and yet trypanosomes are present in the blood. This occurs when animals have high tolerance to trypanosome infections, which corresponds to acquired immunity. This is commonly observed in the Maputo area, in Mozambique (Hornby, 1947), where more than 60.000 head of cattle exist in close contact with *G. brevipalpis* and *G. austeni.*

Experiments carried out proved that cattle from tsetse fly free areas introduced in the Maputo region reacted like the local cattle

towards trypanosomiasis. This means that under normal conditions, and in the absence of other diseases, animals recover spontaneously.

We transfered some cattle infected with *T. congolense* or *T. vivax* from Maputo to a *G. morsitans* area with similar conditions as for climate and grazing. All animals died of nagana within few months. This experiment leads us to admit that resistance of Maputo cattle is sufficient for the local strains of *T. congolense* and *T. vivax,* but insufficient for the strains of same trypanosomes transmitted by *G. morsitans.*

The conclusion is that strains of *T. congolense* and *T. vivax* transmitted by *G. brevipalpis* and *G. austeni* in Maputo area are less virulent than those transmitted by *G. morsitans* (de Andrade Silva, 1953).

Before we come to the subject trypanosomiasis control we deem of importance to consider the distribution of domestic animals in relation to the distribution of tsetse fly.

There are vast areas where the fly exists and where no domestic animals are kept, with the exception of perhaps a few goats. Africans in some of these areas never possessed cattle. Game and pastures are abundant.

In other areas which are fly-belts today, cattle was wiped out by nagana in result of the tsetse advance.

In other areas, however, cattle exists exposed to contact with tsetse fly. Besides the Maputo area already mentioned here are over 50,000 head of cattle in the Sabie district and also in other areas infested by *G. austeni.*

In other areas cattle during the dry season is bitten by *G. morsitans* or *G. pallidipes* when moving in search of new pastures.

Obviously there is a great number of cattle in tsetse fly free areas; sometimes so many as to cause soil erosion.

Again there are other tsetse free areas where cattle does not exist: sometimes this is due to lack of water, grazing, or due to agriculture.

In other areas Africans are not interested in cattle or do not have the economic means for buying.

Distribution of cattle is largely subject to the habits and aptitudes of the native population. There are traditionally pastoral tribes whose lives are closely interwoven with their cattle and it amounts to

tragedy for these natives to see their cattle destroyed by the advancing tsetse fly. Other tribes are agricultural and to introduce cattle is very difficult because these natives are not used to cattle and are not interested.

The treatment of animal trypanosomiasis with the new and powerful tripanocides makes it possible these days to keep cattle in tsetse fly areas, including *G. morsitans* and *G. pallidipes,* provided the fly density is low.

Periodical treatment remains necessary. This incurs great expenses: veterinarian assistance, costly drugs and so on. Naturally all these precautions are not sufficient and do not dispense with the necessity of eradicating the fly itself from certain areas.

Amongst the trypanocides we mention the dimidium bromide, a phenanthridinium compound, which since 1946 was in extensive use.

*T. congolense* infections are easily cured and its action on *T. vivax* is also favourable.

It is rather toxic. Photosensitization, a serious complication, is more frequent in some areas.

Dimidium bromide has prophylactic action against *T. congolense* and *T. vivax,* but the period of protection is short.

It was the most widely used drug in Mozambique for some years.

Antrycide appeared less than ten years ago. The antrycide salts have different action: the methylsulphate is easily absorbed and curative, and the chloride, slowly absorbed, is chiefly prophylactic.

The trypanocide action of antrycide has many possibilities. It is very active against *T. congolense* and *T. vivax* and in a lesser degree against *T. simiae* and *T. brucei.* Antrycide is less toxic than dimidium bromide.

Methylsulphate is in extensive use in the treatment of nagana and the results are excellent.

The mixture of methylsulphate and chloride known as antrycide pro-salt is used prophylactically. Its preventive action is notable, but it depends on the density of the fly.

A drawback of antrycide is the fact that it can produce resistant strains of trypanosomes especially where used as prophylactic.

The period of protection is estimated at two months and in tsetse control operations antrycide is of great value.

Ethidium bromide, also a phenanthridinium compound, is a good trypanocide against *T. congolense* and in lesser degree to *T. vivax*. In Mozambique we have little experience with this drug, but such trials as we made lead us to the conclusion that it is less toxic than dimidium bromide.

Berenil, a diamidine, was experimented in Mozambique for the first time in 1953, and a good curative action against *T. congolense* infections was established. In other territories it was found to be also active against *T. brucei* and *T. vivax*.

In further experiments we found that berenil was active against antrycide resistant strains of trypanosomes.

Prothidium used in various countries protected cattle kept in fly areas against *T. congolense, T. vivax* and *T. brucei* for the period of 6 to 8 months. We have just started using this drug in Mozambique.

Experiments with suramin complexes (J. Williamson, 1956, and R. S. Desowitz, 1957) showed that these drugs give a protection for periods over 8 months.

Suramin (Bayer 205, antrypol, moranyl) can be mixed with any of the above mentioned basic trypanocides.

The toxicity of the suramin complexes is less than that of the basic trypanocide.

I have limited myself to mention only the most used drugs for the control of animal trypanosomiasis.

I am of the opinion that chemoprophylaxis, today, gives us great hopes of better future for cattle breeding in Africa.

Dr. P. Mornet, in his paper, will give us later on, in this Congress, a detailed account on treatment and chemoprophylaxis of animal trypanosomiasis.

## TSETSE CONTROL

The valuable research work on the biology and ecology of some species of glossina, chiefly undertaken by British scientists, gave us various methods of tsetse control. Some of these methods are applicable, others are largely theoretical. But all these methods have proved rather expensive and the results are too slow.

As the various species of glossina show different preferences as to climatic conditions, vegetation and feeding habits, it may happen

that a method of control is efficient against one species, giving poor results with other species or again in others may prove totally inefficient.

It can also happen that a good control method cannot be put in practice owing to local conditions.

We have mentioned already that the highly useful trypanocides in use these days against sleeping sickness and nagana have changed the aspect of these diseases, but we must again stress the point that drugs only are not sufficient. To finally elliminate trypanosomiasis the eradication of tsetse fly itself is essential.

Local conditions and factors greatly influence the methods of control, some of which, although not directly related with the insect, can impose and lead to different measures against the fly. Sometimes these local conditions and factors even make tsetse operations void and ineffective.

Deciding on tsetse control we always have to take into consideration the economic policy of the territory concerned, the pressing necessity of land owing to the increase of the population, the lack of cattle in areas of good pastures and such development plans as may be under consideration or already in progress.

Our decision to start tsetse control rests chiefly on the above mentioned factors, and besides there are others.

Density of population is an important condition in the tsetse control. Scarcity of population in East Africa is related with the prevalence of *G. morsitans*. If there are not enough people to settle in the areas reclaimed from the tsetse fly, consolidation will be expensive and difficult, almost impossible: the fly will return and all efforts are in vain.

In Mozambique, normally we only control the tsetse in areas suitable for development and settlement. For this evidently we need at least good soil and enough water.

We also fight the fly in cattle areas where cattle breeding has a fair chance of success and also where tsetse is encroaching and threatning other areas.

If the tsetse control is expensive, the costs of settlement and occupation of reclaimed areas are even more so: water supply, clearing and preparing the land for agriculture, road building, housing, etc.

I cannot describe in particular all the methods of tsetse control nor the work involved by them, and therefore I shall mention only the most important ones.

I leave it to Dr. Ford, here present, to tell you about this subject in detail.

Amongst the *methods of direct attack* on the tsetse fly, *insecticides* have proved efficient in confined and not very large areas.

Insecticides sprayed by plane resulted in eradicating *G. pallidipes* from reserves and other areas in Zululand. Those areas were usually isolated beforehand by total clearing from the surrounding contry.

In order to check sleeping sickness epidemics and to control *G. palpalis,* insecticides have been spread over riverine forest with good results.

In Southern Rhodesia the use of insecticides against *G. morsitans* was discontinued because it proved too expensive, and also because of bad weather conditions which limited the flying time.

I am convinced that the use of insecticides either sprayed by aircraft or on ground will increase in the future.

As for the *methods of indirect attack,* I mention the *game elimination, change of the vegetation cover* and *human settlement.*

*Game destruction,* as a method of control, acts by starvation deprieving the tsetse fly from its food ressources. This method is in practice in various countries. It is economic and relatively easy to apply. Strict control and good organization are necessary.

In Southern Rhodesia, Jack and Chorley held a long campaign based on game destruction and 10,000 square miles were cleared of *G. morsitans.*

Robertson (1956) reports that by the same method, jointly with human occupation, in Uganda over 6.000 square miles were reclaimed from *G. morsitans* and *G. pallidipes.*

In Mozambique, over 4.000 square miles were gained from *G. morsitans* through game destruction either by itself or jointly with human occupation, since 1947.

This method of control is unpleasant because the fauna, one of the most interesting and characteristic assets of Africa, is being destroyed.

It is under discussion in order to obtain the same results against tsetse fly if it would be sufficient to destroy only the bigger species instead of indiscriminate destruction.

The important paper of Weitz (1956) on the identification of blood specimens taken from numerous flies belonging to various species of glossina is a valuable contribution to the knowledge of the feeding preferences of each species.

*G. morsitans,* although the blood of other animals was identified, showed preference for wart-hog and wild pig.

We have to take into consideration that the tsetse fly, when its favourite animals are not present, feeds on others. According to local conditions and seasonal changes, the fly must feed on the most readily available animals.

In Mozambique, with the exception of animals under protection of the law, we destroy all mammals big and small, as we have found that selection as the one advised by Potts (1952) does not give the desired results.

*Deforestation* is another method of indirect attack against tsetse fly by transformation of its habitat. Total clearing comes out too expensive. It was only applied to stop the advance of tsetse fly, especially of the *morsitans* group.

*Discriminative clearing* gives good results against some tsetse fly species. In Mozambique this method has proved valuable against *G. austeni;* and also against *G. morsitans* in conjunction with game ellimination.

This method is applied successfully in various East African territories and the proportion of bush destroyed varies from 3 to 10 percent.

Other ways of partial clearing have been described which are less expensive than discriminative clearing.

*Human occupation* consolidates the results obtained by other methods of control, and even on its own can be responsible for the disappearance of tsetse fly, provided it is of a sufficient density, principally due to agriculture.

As has been mentioned, it is sometimes necessary to combine methods. For instance during operations against *G. morsitans* based on game destruction, we may find vegetation clearing necessary on

the residual areas. The combination of the human occupation with any other method of control is, as a rule, very successful.

It is absolutelly necessary to have good planning for the settlement of the population, in order to avoid difficult problems for the future. Africans will have to work land better, and overstocking of cattle must be avoided in the area. Good agricultural measures and the control of cattle will prevent soil erosion.

The cooperation of the population is of the greatest interest. A better future will be assured if the population accepts the recommended agricultural and cattle breeding methods in areas where tsetse operations are in progress.

The different attitude taken by the Africans as far as initiative and cooperation are concerned could explain contrasts of the tsetse control results from one area to the other. Results are good when people show initiative, work hard and are generally interested in what is being done for them. Vice versa results are bad when people are suspicious, recalcitrant and show lack of collaboration.

The research work done by the glossinologists is extensive. Among so many valuable papers we will find no doubt, after careful study, scientific basis for new tsetse control methods more practical and less costly than the present ones. Those who want to go into this matter may find it a very rewarding subject.

It is not only the people working directly in trypanosomiasis who could find solutions to these problems, but it is of the utmost importance that the Governments of our different countries also take a hand in this.

Apart from the health problems, our Governments should look into the matter of preparing the evolution of the people, in order that they may learn to work the land better and improve their agricultural knowledge and economy, which would raise the standard of living. The abandon of backward preconceived ideas is only possible through education.

When this comes about one day, the fight against the tsetse fly will come easier and more profitable.

Many of the countries here represented have contributed to lessen the immense problems caused by the tsetse fly. Doctors, laboratory workers, entomologists, veterinarians, and other scientists

and technical people, both in Africa and elsewhere, interested in the diseases carried by tsetse fly or in the fly itself have done invaluable work.

New scientific knowledge and the continued efforts of all of us no doubt will gradually lift this black cloud over Africa, which would result in enormous surge forward in the progress and the economic development of this great continent.

## BIBLIOGRAPHY


APTED, F. A. C. — «Four years' experience with melarsen oxide/B.A.L. in the treatment of late-stage Rhodesian sleeping sickness», *Trans. Roy. Soc. Trop. Med. Hyg.*, Vol. 51, No. 1, 1957.

BURTT, E. — «Incubation of tsetse pupae: increased transmission-rate of *Trypanosoma rhodesiense* in *Glossina morsitans*», *Ann. Trop. Med. Parasit.*, Vol. 40, No. 1, 1946.

DE ANDRADE SILVA, M. A. — «Alguns aspectos epidemiológicos da tripanossomíase rodesiense em Moçambique», *An. Inst. Med. Trop.*, vol. 9, n.° 3, 1952.

DE ANDRADE SILVA, M. A. — *Relatório Anual da Missão de Combate às Tripanossomíases*. Imprensa Nacional, Lourenço Marques, 1953.

DE ANDRADE SILVA, M. A., CASEIRO, A., CARMO, R. P., and BASTO, A. X. — *Arsobal in the treatment of* T. rhodesiense *sleeping sickness*. 5th meeting of I.S.C.T.R., Pretoria. Publication No. 206, B.P.I.T.T. Léopoldville, 1954.

DE ANDRADE SILVA, M. A. — *The value of drugs commonly used in the treatment of* T. rhodesiense *sleeping sickness*. 6th meeting of I.S.C.T.R., Salisbury. Paper 3 (2), 1956.

DE ANDRADE SILVA, M. A., and CASEIRO, A. — *Prophylactic action of diamidines against* T. rhodesiense *nifection*. 6th meeting of I.S.C.T.R., Salisbury. Paper 3 (1), 1956.

DE BARROS MACHADO, A. — «Revision systématique des Glossines du groupe *palpalis (Diptera)*». *Subsídios para o estudo da biologia da Lunda*. Museu do Dondo. Serviços Culturais da Companhia de Diamantes de Angola. Lisboa, 1954.

DESOWITZ, R. S. — «Suramin complexes. Prophylactic activity against *Trypanosoma vivax* in cattle», *Ann. Trop. Med. Parasit.*, Vol. 51, No. 4, 1957.

FAIRBAIRN, H. — *Sleeping sickness in Tanganyika*. Gov. Printer. Dar es Salaam, 1944.

FAIRBAIRN, H. — «Sleeping sickness in Tanganyika Territory, 1922-1946», *Trop. Dis. Bull.*, Vol. 45, No. 1, 1948.

FAIRBAIRN, H. — «Animal reservoirs of *Trypanosoma rhodesiense* and *Trypanosoma gambiense*», *Ann. Soc. Belge Méd. Trop.*, Vol. 34, No. 5, 1954.

HORNBY, H. E. — «Report on the tsetse fly problems of Maputo», *An. Inst. Med. Trop.*, Vol. 4, 1947.

HORNBY, H. E. — *Animal trypanosomiasis in Eastern Africa, 1949.* Colonial Office. Her Majesty's Station Office, 1952.

MARNEFFE, J. — «Observation d'un foyer de trypanosomiase rhodesiense en Urundi. Historique et aspect géographique. Clinique. Traitement. Prophylaxie. 1954-1955», *Ann. Soc. Belge Méd. Trop.*, vol. 35, n.° 3, 1955.

MORRIS, K. R. S. — «The ecology of epidemic sleeping sickness. I — The significance of location», *Bull. Ent. Res.*, Vol. 42, Part 2, 1951.

NEUJEAN, G. — «Déclin et danger résiduel de la maladie du sommeil à *T. gambiense*», *Ann. Soc Belge Méd. Trop.*, vol. 34, n.° 5, 1954.

POTTS, W. H. — «The Shinyanga game destruction experiment», *Bull. Ent. Res.*, Vol. 43, Part 2, 1952.

ROBERTSON, A. G., and BERNACCA, J. P. — *Game elimination as a tsetse control measure in Uganda.* 6th meeting of I.S.C.T.R., Salisbury. Paper 19, 1956.

VAN HOOF, L. — «Trypanosomiase humaine africaine: reflexions sur la lutte chimique contre l'endemie», *Proceedings of the Fourth International Congress on Tropical Medicine and Malaria.* Washington, 1948.

VAUCEL, M. — *Quelques observations et suggestions concernant la recherche dans la maladie du sommeil. 1ère réunion du I.S.C.T.R.*, Londres. Communication 4, 1949.

WEITZ, B., and GLASGOW, J. P. — «The natural hosts of some species of Glossina in East Africa», *Trans. Roy. Soc. Trop. Med. Hyg.*, Vol. 50, No. 6, 1956.

WILLET, K. C. — *The relationship of* Trypanosoma rhodesiense. 6th meeting of the I.S.C.T.R., Salisbury. Paper 9, 1956.

WILLIAMSON, J. — *Prophylactic activity of suramin complexes in animal trypanosomiasis.* 6th meeting of I.S.C.T.R., Salisbury. Paper 28, 1956.

# SOME OF THE PROTOZOOLOGICAL PROBLEMS OF AFRICAN HUMAN TRYPANOSOMIASIS

K. C. WILLETT

It is clearly impossible in the short span of fifteen minutes to cover more than a very small part of the large subject of the protozoology of trypanosome infections and I have therefore so chosen my title as to impose three important limitations: the first that my remarks are restricted to the pathogenic trypanosomes of *Africa:* the second that I am concerned only with those related to *human* sleeping sickness, and the third that I make no claim to consider more than a few of the problems which seem to me, personally, to be of importance.

The biggest single puzzle still remaining is the exact relationship to each other of the three closely related species of trypanosome *T. brucei, T. gambiense* and *T. rhodesiense,* of which the first cannot infect man though the others both can. Briefly, the problem is to account for the contrast between two sets of facts, the one that *T. brucei* and *T. rhodesiense* resemble each other so closely that *only a direct test of infectivity to man can distinguish the two,* and the other that the single attribute in which *T. rhodesiense* differs from *T. brucei* and has in common with *T. gambiense* — infectivity to man — has grown in importance as knowledge and experience have accumulated until a plausible argument can be put forward that it may be a fundamental property, whose presence or lack is more important than all the apparent resemblances between *T. rhodesiense* and *T. brucei.*

The similarities between *T. rhodesiense* and *T. brucei* need no emphasis. Both are much more virulent to small laboratory animals than *T. gambiense* and will readily infect some species in which

*T. gambiense* takes only with great difficulty or not at all. Both, in infections of small laboratory animals, show periods in which posteronuclear forms are readily found, and both are resistant to tryparsamide. There is, in short, no proven difference either qualitative or quantitative between *T. rhodesiense* and *T. brucei* other than the ability of the former to infect man.

Such a striking similarity naturally suggested that the two might be no more than variants of the same species and led to a search for the conditions under which one might change into the other. The outstanding hypothesis was that of Yorke, Adams and Murgatroyd (1930) that in conditions of malnutrition, or for other reasons, the trypanocidal effect of human serum on *T. brucei* could be lost and that *T. brucei* could then establish itself in man. They postulated that *T. rhodesiense* was a *T. brucei* thus recently established in man and that, if repeatedly transmitted naturally by fly through animals, it would soon lose its infectivity to man and thus have reverted to *T. brucei*. This hypothesis could be tested by three different experiments, all of which have been done, and all have given results conflicting with the expectations of the hypothesis.

From Tinde laboratory in Tanganyika the continued infectivity to man of *T. rhodesiense* transmitted by *Glossina* through animals was reported by Corson (1939) after 5 years, by Fairbairn & Burtt (1946) after 10 years and by Willett & Fairbairn (1955) after 18 years. Now, even after 23 years, the strain nas still not lost its infectivity to man (Ashcroft, M. T., personal communication). The experiment of infecting a man with a strain of *T. rhodesiense* made sensitive to his own serum was performed by Fairbairn (1934) and, taken in conjunction with the failure to infect man with *T. brucei* made resistant to human serum by Collier (1924) and Lester (1933), showed that the susceptibility to human serum was in no way connected with the property of infectivity to man. Thus three critical experiments failed to alter the property of infectivity to man as they might have been expected to do, one of them showing the fixity of the characteristic throughout 23 years in animals — an immensely long time in terms of generations of trypanosomes. Finally, from a study of the early reports from 1907 onwards, Willett (1956) postulated that the virulent sleeping sickness that appeared in Rhodesia in 1908 and afterwards was no more than a further extension, into a new

area and a new species of tsetse fly, of the infection which was known to have been introduced from the northwest (Katanga, Belgian Congo) about 1907.

Whether this was so or whether the Rhodesian form of sleeping sickness was already present in the Luangwa Valley, as suggested by Kinghorn (1925), the unfailing association of *T. gambiense* with the *G. palpalis* group of tsetse fly and of *T. rhodesiense* with the *G. morsitans* group remains to be satisfactorily explained. The present author *(loc. cit.)* drew attention to the consistent association of *G. pallidipes* with appearances of *T. rhodesiense* in the wake of *T. gambiense,* tentatively suggested that transmission through this species might produce the change and also mentioned the possibility that game animals, which play a much larger part as hosts of the *G. morsitans* group than of the *G. palpalis* group, might be the responsible agency. Ashcroft (personal communication) has pointed out the marked effect of the host animal on the trypanosome as shown at Tinde (Fairbairn & Burtt, 1946; Willett & Fairbairn, 1955; Ashcroft, M. T., in press), favouring the view that the mammalian host rather than the species of insect vector is much more likely to effect such a change; in fact it may be that no changing of the characteristics of trypanosomes takes place at all, but that the wild animals merely act as a filter and select only the more virulent strains, the less virulent *T. gambiense* being incapable of establishing or maintaining itself in animals which have a marked resistance even to the virulent *T. rhodesiense.*

In brief, there are two main problems of the relationship between the trypanosomes of the *T. brucei* group; the first is to determine what causes infectivity to man and the second is to account for the constant association of types of trypanosomes with types of tsetse flies. The ability or inability to infect man must be the result of a metabolic difference in the trypanosomes and seems to be essentially a problem for the biochemist. Almost as much, however, the second problem seems to be one for the biochemist for two of the characteristics separating the virulent *T. brucei* and *T. rhodesiense* from the less virulent *T. gambiense* must also represent biochemical differences, quantitative if not qualitative, namely the resistance to tryparsamide and greater virulence to small laboratory animals. A further difference between *T. rhodesiense* and *T. gambiense* has been described by

Fairbairn & Culwick (1949), in the length of the metacyclic forms, and the last feature distinguishing again both *T. brucei* and *T. rhodesiense* from *T. gambiense* is the frequency of occurrence (usually in infected small laboratory animals) of posteronuclear forms.

This brings me to what I consider a second great problem of the protozoology of the trypanosomes of the *T. brucei* group and that is the explanation of their remarkable polymorphism. Robertson (1912, 1912*a*, 1913) studied *T. gambiense* and *T. brucei* in monkeys and concluded that the short (now called stumpy) forms elongated through the intermediade to the long (now called slender) stage in which division occurred to reform directly or indirectly the stumpy forms. Fairbairn & Culwick (1946) concluded from measurement and statistical analysis that the stumpy, intermediate and slender forms were distinct entities genetically different. Very recently Wijers (1957) and Ashcroft (1957) have independently reached conclusions which are remarkably similar — Wijers that «stumpy forms are produced by shrinkage of slender forms in circumstances in which trypanosomes have to try to adapt themselves to an altering, or altered, environment» and Ashcroft that «the stumpy, including the posteronuclear, trypanosomes are formed from the slender forms by the action of antibodies; they rarely divide and most of them are destroyed».

These conclusions are consistent with the observations of many other workers that high proportions of stumpy forms are associated with rapidly decreasing numbers of trypanosomes in the general circulation, but no attempt is made by either worker to explain the meaning of the posteronuclear form, which is a matter of importance so long as the appearance of this form is given any diagnostic significance. The occurrence of the posteronuclear form in infections with *T. brucei* and *T. rhodesiense* rather than *T. gambiense* suggests an association with the virulence of the strain, but in the most virulent strains parasitaemia increases rapidly to the death of the animal within a short time and in these circumstances few stumpy forms are found and posteronuclear forms are absent or very rare. On the other hand, in a relapsing infection stumpy forms are most abundant when the parasitaemia is decreasing, and the data given by Ashcroft *(loc. cit.)* in his Table 1 show that the greater the proportion of stumpy forms the greater will be the proportional decrease in parasitaemia within

the next 24 hours (P 0.001). The same data further show that as the percentage of stumpy forms increases so the percentage of posteronuclear forms increases more rapidly than in linear proportion. Regression of his percentages of posteronuclear forms on percentages of stumpy forms gives the following mean corresponding proportions:

| Percentages of stumpy (including posteronuclear) | Percentages or posteronuclear |
|---|---|
| 20 | 4 |
| 40 | 11 |
| 60 | 24 |
| 80 | 42 |

with curvilinearity of regression with a probability of less than 0.01. It is therefore possible that the influence that produces short stumpy forms gives rise, as it acts more powerfully, to more and more posteronuclear forms.

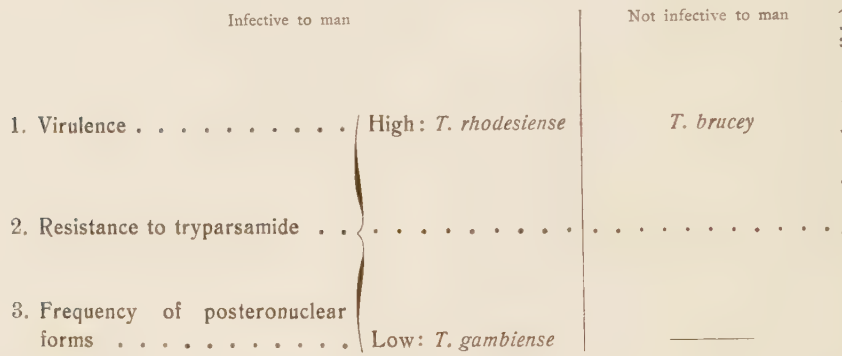
The hypothesis of Lavier (1927) that the posteronuclear form is one of the progeny of a divided stumpy form is open to objections. In the first place dividing stumpy forms are always rare and never increase in number to the extent that posteronuclear forms do. Secondly the hypothesis can hardly account for the numbers of posteronuclear forms exceeding half the stumpy forms, but even the mean figures in the regression given above show that this proportion can be exceeded and Ashcroft records one observation of the percentage of stumpy forms reaching 82, comprising 59 percent posteronuclear and 23 percent «normal».

The most likely conclusion appears to be that the posteronuclear form is the extreme condition of the stumpy form, perhaps — if Ashcroft's belief is true that most of the stumpy forms ar destroyed — it is the stumpy form *in extremis*.

Finally, on the question of this polymorphism, we know that prolonged passage of a strain of the *T. brucei* group through laboratory animals such as rats, mice and guinea pigs will cause not only loss of polymorphism, but also loss of transmissibility by tsetse fly and of resistance, if originally present, to some drugs, notably

arsenicals. The relation of these properties to each other is an important problem and again one in which our most probable source of help would seem to be the biochemist.

A third group of problems that I would class as of great importance is that of identification of trypanosome species, or biological races of species (Hoare, 1943), whatever they be called. Let me return briefly to the relationships of *T. brucei, T. rhodesiense* and *T. gambiense*. They may be summarized in the diagram:




from which it is seen, as I have previously pointed out, that infectivity to man is a criterion of major diagnostic importance. At present we have nothing which can indicate the presence or absence of this property except a direct test on a man. In fact, without trials on humans neither can a virulent strain isolated from a tsetse fly or an animal be positively identified nor can it be determined whether an avirulent strain similarly obtained in a *T. gambiense* area is the form of *T. brucei* which might be expected to fill the fourth cell in the above diagram. An alternative test to indicate whether or not a strain is infective to man is an urgent need. Soltys (1957) has just published serological results which, if they apply consistently to all strains of *T. rhodesiense, T. gambiense* and *T. brucei,* will constitute a major advance in diagnostic methods available for they would achieve this objective of diagnosis without having to expose men to the risk of infection.

Another obvious deficiency in our ability to distinguish strains of trypanosomes is that there is no means, as yet, of carrying the

identification of trypanosomes found in tsetse flies beyond the general group to which they belong.

And finally, thinking of the trypanosome while in the tsetse fly brings another group of major research problems to mind, which can be stated very briefly. Infections of tsetse flies with the *T. brucei* group of trypanosomes are relatively rare; when everything possible is done in the laboratory to obtain such infections, the successes rarely exceed a very small percentage and in the field the proportion of flies so infected is usually of the order of 1 in 500 to 11 in 5,000. Why is it that so few flies become infected with these trypanosomes? Is it not possible that this provides a vulnerable point at which to break the cycle of transmission? A result which showed us either how to decrease or how to increase the frequency of infection would be of value, the former as a step towards control and the latter as making it easier to maintain laboratory strains by fly in order to minimize or prevent the great changes which take place in the usual maintenance by syringe-passage in small laboratory animals.

## SUMMARY

The paper deals with only a selection of problems which seem to the author to be of importance to our knowledge of sleeping sickness.

The opinion is expressed that the distinguishing criterion of infectivity to man, separating *T. rhodesiense* and *T. gambiense* on the one hand from *T. brucei* on the other, is probably more important than all the apparent similarities between *T. rhodesiense* and *T. brucei,* and that we need to understand its cause and to be able to determine whether or not a strain is infective to man without resort to direct tests. Also, if this distinction is so important, we need to know why *T. rhodesiense* and *T. brucei* otherwise resemble each other so closely.

A second problem considered is that of the polymorphism of the *T. brucei* group of trypanosomes; various workers opinions are briefly considered and some new evidence given on the significance of the posteronuclear form suggesting that it may be an extreme example of the stumpy form and that it is not of any great diagnostic significance.

Finally attention is drawn to the impossibility of carrying diagnosis of a trypanosome in the tsetse fly beyond the general group to which it belongs and to the desirability of extending our knowledge of the factors governing infection of the tsetse fly, particularly by the *T. brucei* group trypanosomes, either to decrease infection rates as a step towards control or to increase them to facilitate laboratory research.

## BIBLIOGRAPHY


Ashcroft, M. T. — «The polymorphism of *Trypanosoma brucei* and *T. rhodesiense*, its relation to relapses and remissions of infections in white rats, and the effect of Cortisone», *Ann. trop. Med. Parasit.*, **51**: 301, 1957.

Collier, W. A. — «Ueber einen Versuch, Tsetsetrypanosomen durch Festigung gegen Menschenserum menschenpathogen zu machen», *Arch. Schiffs. Tropenhyg*, **28**: 484, 1924. (Quoted from *Trop. Dis. Bull.*, **22**: 145).

Corson, J. F. — «A fifth note on the infectivity to man of a strain of *Trypanosoma rhodesiense:* three further passages through antelopes and tests on man; two charts of the whole experiment», *J. trop. Med. Hyg.*, **42**: 5, 1939.

Fairbairn, H. — «Experimental infection of man with a strain of *Trypanosoma rhodesiense* apparently susceptible to normal human serum *in vitro*», *Ann. trop. Med. Parasit.*, **27**: 251, 1933.

Fairbairn, H., & Burtt, E. — «The infectivity to man of a strain of *Trypanosoma rhodesiense* transmitted cyclically by *Glossina morsitans* through sheep and antelope: evidence that man requires a minimum infective dose of trypanosomes», *Ann. trop. Med. Parasit.*, **40**: 270, 1946.

Fairbairn, H., & Culwick, A. T. — «A new approach to trypanosomiasis; with a statistical analysis by F. L. Gee», *Ann. trop. Med. Parasit.*, **40**: 421, 1946.

Fairbairn, A., & Culwick, A. T. — «The differentiation of the polymorphic trypanosomes», *Ann. trop. Med. Parasit.*, **43**: 90, 1949.

Hoare, C. A. — «Biological races in parasitic protozoa», *Biol. Rev.*, **18**: 137, 1943.

Kinghorn, A. — «Human trypanosomiasis in the Luangwa Valley, Northern Rhodesia», *Ann. trop. Med. Parasit.*, **19**: 281, 1925.

Lavier, G. — «The posterior-nuclear form», *Interim. Rep. League of Nations Comm. Hum. Trypanosomiasis*, p. 118. Geneva, 1927.

Lester, H. M. O. — «The characteristics of some Nigerian strains of polymorphic trypanosomes», *Ann. trop. Med. Parasit.*, **27**: 361, 1933.

Robertson, M. — «Notes on the life-history of *Trypanosoma gambiense* with a brief reference to the cycles of *Trypanosoma nanum* and *Trypanosoma pecorum* in *Glossina palpalis*», *Philos. Trans., B*, **203**: 161, 1912*a*. *(Rep. S.S. Comm. roy. Soc.*, **13**: 119).

Robertson, M. — «Notes on the polymorphism of *Trypanosoma gambiense* in the blood and its relation to the exogenous cycle in *Glossina palpalis*», *Proc. roy. Soc., B*, **85**: 527, 1912*b*. *(Rep. S.S. Comm. roy. Soc.*, **13**: 94).

Robertson, M. — «Notes on the behaviour of a polymorphic trypanosome in the blood-stream of the mammalian host», *Rep. S.S. Comm. roy. Soc.*, **13**: 111, 1913.

Soltys, M. A. — «Immunity in trypanosomiasis. II. Agglutination reaction with African trypanosomes», *Parasitology*, **47**: 390, 1957.

Wijers, D. J. B. — «Polymorphism in human trypanosomiasis», *Nature*, Lond., **180**: 391, 1957.

WILLETT, K. C. — «The relationships of *Trypanosoma rhodesiense*», paper to the 6th Meeting of the International Scientific Committee for Trypanosomiasis Research, held at Salisbury, S. Rhodesia, September, 1956.

WILLETT, K. C., & FAIRBAIRN, H. — «The Tinde Experiment: a study of *Trypanosoma rhodesiense* during eighteen years of cyclical transmission», *Ann. trop. Med. Parasit.*, **49**: 278, 1955.

YORKE, W., ADAMS, A. R. D., & MURGATROYD, F. — «Studies in chemotherapy. II. The action *in vitro* of normal human serum on the pathogenic trypanosomes, and its significance», *Ann. trop. Med. Parasit.*, **24**: 115, 1930.

# SOME PROBLEMS OF HUMAN TRYPANOSOMIASIS IN AFRICA

H. FAIRBAIRN

## THE TRYPANOSOME

Van Hoof (1947) found that strains of *T. gambiense* in the Belgian Congo could be divided into three types, according to their virulence and their pathogenicity to man.

1) Trypanosomes of low virulence, which were found to be in the majority in new foci or in epidemic extensions from old areas. In man they produced an infection of slow development and trypanosomes were not numerous in the blood. Enlargement of the lymphatic glands was produced late, as were alterations in the cerebrospinal fluid. These strains were generally easily transmitted by tsetse. «These trypanosomes seem adapted to rapid and widespread extension among the native population even if this is scatered.»

At Tinde Laboratory a strain of *T. rhodesiense* has been transmitted through sheep, by cyclically infected *G. morsitans* for 18 and a half years. Willett and Fairbairn (1955) showed that there had been a cyclical fluctuation in the infectivity to man over a period of at least 13 years (1940 to 1953). There had also been a cyclical fluctuation of the virulence of the strain to sheep. When these two factors were considered together, it was found that the infectivity to man had varied in inverse proportion to the virulence of the strain to sheep (though lagging 1-2 years behind), i.e., the lower the virulence of the strain to sheep the higher the infectivity to man.

These observations are confirmatory to those of van Hoof, and they raise some interesting problems.

2) In van Hoof's second category were strains of medium virulence, which killed guinea pigs in 3 to 4 months, with heavy blood infection during the latter weeks. The clinical course of the infection in man, though not malignant, was characterized by frequent relapses and by the appearance of altered cerebrospinal fluid 6 months or a year after the infective bite.

3) Thirdly, very virulent strains of trypanosomes were met with sporadically in all epidemic areas, strains which recalled in man and the guinea pig the evolution of *T. rhodesiense*, by the heavy blood infection and the rapid clinical progress of the disease. In man nervous changes were evident often after 2 or 3 months. The trypanosomes were very resistant to tryparsamide, and were only feebly or not all capable of fly transmission (by *G. palpalis*), especially in the case of relapse after treatment.

Lester (1933), working in Nigeria, examined 7 strains of *T. gambiense* from Gadau, an epidemic area where *G. tachinoides* was the chief vector, and 6 strains from Ayu, an endemic area where the disease appeared to be clinically of a very mild type and individual cases gave histories of long duration without showing any signs of nervous involvement, and where the vector was *G. palpalis*. He examined these strains for virulence in laboratory animals, the occurrence of posterior-nuclear forms, their reaction to tryparsamide and their reaction to human serum *in vitro*.

He found that strains Gadau 6 and Ayu 6 appeared to have the characteristics of *T. rhodesiense*, that strains Gadau 1, 2, 4 and 7 and Ayu 7, 9 and 11 had the characteristics of *T. gambiense*, while the position of strains Gadau 3 (and 5?) and Ayu 5 and 8 was not quite clear. He stated that (in Nigeria) not only do strains occur having all the characteristics ascribed to *T. rhodesiense*, but that there were also a whole series of strains with characteristics between *T. rhodesiense* and *T. gambiense*.

In Sierra Leone, wide variations in the behaviour of various strains of *T. gambiense* have been observed (Hutchinson, 1954). In the country as a whole the cases of sleeping sickness exhibited the usual chronic type of *T. gambiense* infection. In the Kissi and Kono epidemic area, however, classical advanced symptoms and signs were encountered in less than 5 percent of the cases. That the strain was not avirulent was shown by the mortality amongst untreated cases

and by the depopulation of the area. Cases appeared to succumb to a toxaemic type of the disease after a short period, a rapid breakdown occurring before typical advanced clinical symptoms had developed. In the worst-affected areas about 25 percent of the cases were diagnosed by the detection of trypanosomes in stained thick films.

At Fuero, within the above epidemic area, the strain of *T. gambiense* was even more interesting. A high proportion of cases were symptomless, and a large number had no enlargement of the cervical lymph nodes; comparatively few (even of those with some glandular enlargement) showed trypanosomes in fresh gland-juice, while trypanosomes were unusually abundant in the blood of a proportion of cases. In spite of the absence of gross symptoms, over 60 percent of cases showed changes in the cerebrospinal fluid, as compared with 48 percent in survey cases from the Kissi area during the same period (Hutchinson, 1954; also Harding and Hutchinson, 1948). Observations showed that this atypical strain was very sensitive to tryparsamide, but was resistant to normal human serum, which was consistent with the behaviour of *T. gambiense*. Virulence in the local community appeared low (but a European infected in the area had an acute infection).

In 1956 some cases of sleeping sickness in Kano Province, Northern Nigeria, were treated with berenil and others with pentamidine, and they were re-examined after 8 months and one year. Hutchinson (personal communication) wrote: «There was quite a high relapse rate even though all cases had had normal cerebrospinal fluid readings before treatment. The disturbing thing was the very rapid clinical deterioration, from a symptomless, apparently fit individual when last seen (8 months), to the extremely sick patient (at the 12 months' re-examination).»

Fairbairn and Culwick (1949) measured the metacyclic trypanosomes of 2 strains of *T. rhodesiense* and of a recently isolated strain of *T. gambiense* from the Belgian Congo, the strains being transmitted by *G. morsitans*. They found that the mean length of the *T. rhodesiense* strains was 17.36 $\mu$, while that of the *T. gambiense* strain was 15.24 $\mu$. The difference in mean lengths was statistically highly significant; and they therefore suggested that by measuring the metacyclic trypanosomes *T. rhodesiense* could be differentiated from *T. gambiense*.

The work was continued in Vom, with a strain of *T. rhodesiense* from Bechuanaland (for the history of this strain see Fairbairn and Godfrey, 1957) and 4 further strains of *T. gambiense* from various areas of Northern Nigeria, all the strains being transmitted by *G. palpalis*. The results are summarized below:

| Strain | Number of metacyclics measured | Mean length $\bar{x} \pm S\bar{x}$ |
|---|---|---|
| 1. *T. rhodesiense* (Tinde and Narok) | 3,358 | $17.37 \pm 0.027$ * |
| 2. *T. rhodesiense* (Bechuanaland) | 868 | $17.39 \pm 0.048$ |
| 3. *T. gambiense* (Belgian Congo) | 1,887 | $15.35 \pm 0.031$ * |
| 4. *T. gambiense* (Bakundi) | 1,942 | $15.30 \pm 0.038$ |
| 5. *T. gambiense* (Beli) | 2,630 | $16.24 \pm 0.034$ |
| 6. *T. gambiense* (No. 1131) | 200 | $16.20 \pm 0.097$ |
| 7. *T. gambiense* (Kudandan) | 958 | $16.95 \pm 0.044$ |

* Fairbairn & Culwick, 1949.

$S\bar{x}$ is the (estimated) standard error of the mean $\bar{x}$.

The results have been examined by Mr. J. N. Darroch, Department of Mathematics, University of Cape Town. He found that the *T. gambiense* measurements fell into 3 groups (i.e., strains 3 and 4; strains 5 and 6; and strain 7), that the mean length of the metacyclic trypanosomes of each group was highly significantly different from those of the other 2 groups, and that the mean length of strain 7 was still highly significantly different from that of *T. rhodesiense* (group 1 and 2).

Willett (1956), discussing the origin of *T. rhodesiense*, considered that it was a virulent form of *T. gambiense*, which arises whenever the latter is introduced into a *morsitans* area, particularly an area where *G. pallidipes* is present. He suggested two possible explanations for the origin of *T. rhodesiense:*

a) When *T. gambiense* is transmitted by the *morsitans* group of flies, particularly by *G. pallidipes*, it is converted to *T. rhodesiense*. He remarks: «One association seems to me so constant that it deserves careful attention. In all regions where *T. rhodesiense* has appeared in the wake of a previous *gambiense* infection, *G. pallidipes* has been

present.» Corson (1938, 1939*a*, 1939*b*) transmitted *T. gambiense* by *G. morsitans* for over 4 years, and found no alteration in the character of the trypanosome. I have transmitted *T. rhodesiense* for more than 3 ½ years by *G. palpalis,* and again there was no alteration in the trypanosome (Fairbairn and Godfrey, 1957). These periods may be too short; but they at least suggest that it is problematical whether the fly vector has any influence on the characteristics of a strain of trypanosomes.

*b*) Willett's second suggestion is that only the virulent *rhodesiense*-like strains of *T. gambiense* are readily transmissible by the *morsitans* group, and are thus selected. This would imply that *T. gambiense* is really heterozygous, and that van Hoof's virulent strains, which he found to be feebly transmissible, or non-transmissible, by *G. palpalis,* are those which are easily transmitted by the *morsitans* group.

That *T. gambiense* may be heterozygous is an interesting thought. Is the idea supported by the epidemiological observations, and by the measurements of the metacyclic trypanosomes, reported above?

## TRANSMISSION OF THE DISEASE

In the Upper River Division of the Gambia, Hutchinson (1953) found that cases of sleeping sickness *(T. gambiense)* were associated with water holes and river banks, the permanent dry-season haunts of *G. palpalis.* In the intervening countryside *G. morsitans* was widespread, but there was no epidemiological evidence that it was transmitting the disease. Why was this, as it has been shown in the laboratory (Corson, 1939*b*) that *G. morsitans* can transmit *T. gambiense?*

On the other hand, we have had great difficulty in transmitting *T. gambiense* by *G. palpalis.* The flies we used were probably *G. palpalis palpalis.*

In order to explain the epidemiology of *T. gambiense* infections, it is necessary to know the laboratory conditions which will produce maximum infection rates in the tsetse fly. Are *G. palpalis palpalis, G. p. fuscipes* and *G. p. martini* equally involved in epidemics of sleeping sickness? And are similar laboratory conditions (temperature,

humidity, etc.) required for producing high infection rates in each of these species? This problem should receive high priority in any research programme.

## FOOD SUPPLY OF THE TSETSE

Another important point in the epidemiology of trypanosomiasis is the food supply of the tsetse flies, and the question of animal reservoirs of the human trypanosomes. In an important paper Weitz and Glasgow (1956) examined 1,433 blood meals from 7 species of *Glossina* caught in different areas of East Africa. Their results were as follows:

1) *G. morsitans* and *G. swynnertoni* derived about half their food supply from species of *Suidae*, warthog being by far the most important host. «At Daga-Iloi (Tanganyika Territory), there was a definite excess of warthog feeds over those expected from the relative frequency of the host species, for here *Suidae* were responsible for 10 percent of the spoor, but 60 percent of the identified blood meals.»

Rhinoceros was invariably used in the areas in which it was present; while blood from man and domestic animals, although not natural hosts, were always found.

*a*) The animals commonly bitten were roan antelope, reedbuck, buffalo, kudu, bushpig, bushbuck, elephant, giraffe (hippopotamus?).

*b*) The animals rarely bitten were eland, duiker (not fed on by *G. swynnertoni*), waterbuck, impala, baboon, monkey, dogs and cats, hyaena, porcupine and birds.

*c*) The animals *never* bitten were hartebeest, topi, zebra and wildebeest.

2) *G. pallidipes:* Bushbuck was much the preferred host, and bushpig was the second choice. These flies had not fed on buffalo, duiker or waterbuck.

3) *G. austeni:* «The flies from Zanzibar appear to be completely dependent on bushpig.» They had not fed on duiker, and contained no human blood.

4) *G. longipennis:* In the few flies examined, all caught at these same place, they had all fed on rhinoceros. They had not fed on buffalo and bushbuck nor did they have human blood.

5) *G. brevipalpis:* About 60 percent of the flies had fed on hippopotamus, while 26 percent had fed on *Suidae*, chiefly bushpig, and 10 percent ou bushbuck. They had not fed on buffalo, reedbuck, duiker nor waterbuck, nor did they have human blood.

While 13 out of 281 *G. morsitans* had fed on baboon, and a single feed was recorded from *G. swynnertoni*, the other species did not feed on baboon. Only *G. morsitans* was found to feed on monkey.

The laboratory evidence that a large number of animals (including some of those mentioned above) and birds can be infected with *T. rhodesiense* has been reviewed by Fairbairn (1954). The food supply of *G. morsitans, G. swynnertoni* and *G. pallidipes* reinforces the epidemiological evidence (Fairbairn, 1948, and Jackson, 1955) that antelope can, and probably do, act as reservoirs of *T. rhodesiense*.

6) *G. palpalis:* In East Africa, Weitz and Glasgow found that in one area *G. palpalis* fed mainly on reptiles, but that in another area a large proportion of feeds were of avian origin, with bushbuck as the source of at least some meals. «The preference for bushbuck is noticeable, but it was the only bovid there except for duiker and eland which each provided one meal.»

Little or no work has been done in West Africa on the food supply of *G. palpalis* and *G. tachinoides*. Nash and Page (1953), by observing the frequency of animals seen, came to the conclusion that *G. palpalis* could thrive on a diet of only baboon, duiker, monitor lizard and possibly monkey blood. The fallacy, however, of this observational method is shown by the fact that *G. morsitans* and *G. swynnertoni* did not feed on hartebeest, topi (two very closely related species), zebra and wildebeest, and only very occasionally on impala, although all these animals are seen regularly and in numbers in the fly areas; and several of the other species did not feed on duiker. As Buxton (1955) remarks: «In considering evidence from association, the human being, who depends so little on a sense of smell, must not make the error of thinking that because he cannot see animals the tsetse could not *find* them.»

*G. palpalis* and *G. tachinoides* transmit both *T. congolense* and *T. vivax* (Lloyd and Johnson, 1924; Lloyd *et al.*, 1924; Squire, 1951; Nash and Page, 1953), so that these flies must feed on some mammal which acts as a reservoir for these trypanosomes.

Van Hoof (1947) had no doubt of the survival of *T. gambiense* by a chain of transmissions from tsetse flies to domestic animals. Hoare remarked, in discussing van Hoof's paper, that there had been a growing tendency to deny the existence of animal reservoirs of *T. gambiense,* and to regard man as the only source of infection to the tsetse fly. One of the reasons for this is probably the difficulty of detecting *T. gambiense* in the blood of animals, a point emphasised by van Hoof and his colleagues.

If, however, *G. palpalis* and *G. tachinoides* are infected with *T. congolense* and *T. vivax* by feeding on mammals, then it is probable that they also transmit *T. gambiense* to the same mammals which would, or could, act as reservoirs of the human disease. The problem is: what are the mammals? This can only be decided when a large amount of work is done to find out what are the real food supplies of these species of flies in West Africa. In view of the periodic waves of *gambiense* infections that occur, it is difficult to believe that infection is solely a man-fly-man one.

## BIBLIOGRAPHY


Buxton, P. A. — *The natural history of tsetse flies.* London School of Hygiene and Tropical Medicine, Memoir No. 10, London, H. K. Lewis & Co. Ltd., 1955.

Corson, J. F. — «A third note in a strain of *Trypanosoma gambiense* transmitted by *Glossina morsitans*», *Ann. Trop. Med. Parasit.*, **32**: 245, 1938.

Corson, J. F. — «A final note on a strain of *Trypanosoma gambiense* transmitted through monkeys by *Glossina morsitans*», *Ibid.*, **33**: 91, 1939a.

Corson, J. F. — «A summary of the work of the Research Scheme on *Trypanosoma rhodesiense* during the years 1930 to 1938», *East Afr. Med. J.*, **16**: 84, 1939b.

Fairbairn, H. — «Sleeping Sickness in Tanganyika Territory, 1922-1946», *Trop. Dis. Bull.*, **45**: 1, 1948.

Fairbairn, H. — «The animal reservoirs of *Trypanosoma rhodesiense* and *Trypanosoma gambiense*», *Ann. Soc. Belge. Méd. Trop.*, **34**: 663, 1954.

Fairbairn, H., and Culwick, A. T. — «The differentiation of the polymorphic trypanosomes», *Ann. Trop. Med. Parasit.*, **43**: 90, 1949.

Fairbairn, H., and Godfrey, D. G. — «The local reaction in man at the site of infection with *Trypanosoma rhodesiense*», *Ibid.*, **51**: 464, 1957.

Harding, R. D., and Hutchinson, M. P. — «Sleeping sickness of an unusual type in Sierra Leone and its attempted control», *Trans. Roy. Soc. Trop. Med. Hyg.*, **41**: 481, 1948.

HUTCHINSON, M. P. — «The epidemiology of human trypanosomiasis in British West Africa. I. — The Gambia, with special reference to Upper River Division», *Ann. Trop. Med. Parasit.*, **47**: 156, 1953.

HUTCHINSON, M. P. — «The epidemiology of human trypanosomiasis in British West Africa. III. — Sierra Leone», *Ibid.*, **48**: 75, 1954.

JACKSON, C. H. N. — «The natural reservoir of *Trypanosoma rhodesiense*», *Trans. Roy. Soc. Trop. Med. Hyg.*, **49**: 582, 1955.

LESTER, H. M. O. — «The characteristics of some Nigerian strains of the polymorphic trypanosomes», *Ann. Trop. Med. Parasit.*, **27**: 361, 1933.

LLOYD, LL., and JOHNSON, W. B. — «The trypanosome infections of tsetse flies in Northern Nigeria and a new method of estimation», *Bull. Ent. Res.*, **14**: 265, 1924.

LLOYD, LL., JOHNSON, W. B., YOUNG, W. A., and MORRISON, H. — «Second report of the tsetse fly investigation in the northern provinces of Nigeria», *Ibid.*, **15**: 1, 1924.

NASH, T. A. M., and PAGE, W. A. — «The ecology of *Glossina palpalis* in Northern Nigeria», *Trans. Roy. Ent. Soc.*, **104**: 71, 1953.

SQUIRE, F. A. — «Seasonal variation in the incidence of *Trypanosoma vivax* in *Glossina palpalis* (R.-D.)», *Bull. Ent. Res.*, **42**: 371, 1951.

VAN HOOF, L. M. J. J. — «Observations on trypanosomiasis in the Belgian Congo», *Trans. Roy. Soc. Trop. Med. Hyg.*, **40**: 728, 1947.

WEITZ, B., and GLASGOW, J. P. — «The natural hosts of some species of *Glossina* in East Africa», *Ibid.*, **50**: 593, 1956.

WILLETT, K. C. — «The relationships of *Trypanosoma rhodesiense*», I.S.C.T.R., Paper (6) 9. B.P.I.T.T., 1956.

WILLETT, K. C., and FAIRBAIRN, H. — «The Tinde Experiment: A study of *Trypanosoma rhodesiense* during eighteen years of cyclical transmission», *Ann. Trop. Med. Parasit.*, **49**: 278, 1955.

# O DIAGNÓSTICO LABORATORIAL DA DOENÇA DO SONO

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O diagnóstico laboratorial da doença do sono tem grande importância.

Os riscos de que o tratamento se reveste, a indispensável perseverança no prosseguimento do tratamento quando, por vezes, a sintomatologia é inexistente e o longo período de *contrôle* com exame parasitológico, do líquido e clínico, a que é preciso submeter os doentes são factores que não se devem negligenciar ao fazer um diagnóstico de tripanossomíase. O diagnóstico, para ser seguro, deve alicerçar-se sempre num exame laboratorial que tenha revelado a presença de tripanossomas, visto o quadro clínico e as alterações do líquido, por muito sugestivos que sejam, não serem suficientes para a caracterização exacta da doença.

A pesquisa de tripanossomas nem sempre é fácil. Em doentes com quadro clínico típico a pesquisa pode ser difícil ou negativa durante períodos relativamente longos. Em indivíduos aparentemente sãos podem encontrar-se tripanossomas com facilidade. Embora sejam habitualmente mais fáceis de encontrar no começo da doença, nenhuma lei parece regular as variações de número a que estão sujeitos. Na grande maioria dos doentes encontram-se, porém, ao fazerem-se os primeiros exames — mas estes têm de ser, com frequência, demorados.

Os tripanossomas encontram-se no sangue, na linfa, L. C. R., nos espaços intersticiais dos tecidos e nos derrames.

Podem ser pesquisados em exame directo, por métodos de concentração, em cultura, ou após a inoculação a animais.

No início da doença, quando não se estabeleceu ainda reacção ganglionar, nos casos em que já desapareceu, ou ainda quando os exames de suco são negativos, recorre-se aos exames de sangue — periférico ou venoso — e do líquido cefalorraquidiano.

O exame de sangue a fresco, feito entre lâmina e lamela, pode ser muito útil no começo da doença. Quando dos primeiros acessos febris, os tripanossomas costumam ser numerosos e a sua mobilidade, no exame a fresco, facilita a pesquisa. O exame a fresco serve ainda para se ver se há auto-aglutinação das hematias, muito frequente na doença do sono, mas sem valor específico.

Fora do período inicial da doença as pesquisas de tripanossomas, nas preparações a fresco ou nos esfregaços corados, dão percentagens de exames positivos pequenas se as compararmos com as dadas por outros métodos; por este motivo, são exames pouco usados. É de notar, contudo, que os exames directos de sangue permitiram a Martin e Lebeauf encontrar tripanossomas em cerca de 40 por cento dos doentes gravemente enfermos ou que estavam no período terminal da doença.

De maior valor é o exame de sangue em gota espessa, mas, apesar disso, fora dos períodos epidémicos, dá uma percentagem de exames positivos pequena se a compararmos com a dada pelos métodos de centrifugação.

Enquanto com o processo de gota espessa se podem obter percentagens de cerca de 50 por cento de exames positivos, pelo processo de centrifugação obtêm-se percentagens que excedem 90 por cento.

A gota espessa, contudo, pela sua rapidez de execução e bons resultados que dá, quando pacientemente observada, é processo valioso a que na prática se recorre com frequência, não só para o diagnóstico dum doente de consulta, mas também associada a outros métodos para se isolar a maior parte dos doentes dum núcleo populacional antes de se fazer a quimioprofilaxia.

Os métodos de centrifugação têm grande valor. São de execução relativamente morosa, mas permitem obter percentagens de resultados positivos muito elevadas — 96 por cento (Martin e Lebeauf), 92 por cento (Letonturier e Jamot).

Os processos de centrifugação baseiam-se na diferença de velocidade com que sedimentam os glóbulos, as plaquetas e os tripanossomas. Por os tripanossomas serem mais leves, sedimenta-se a maior

parte dos glóbulos e das plaquetas nas primeiras centrifugações para que no último sobrenadante fiquem quase só tripanossomas. Compreende-se assim a importância que tem o tempo de centrifugação, o número de rotações por minuto da centrífuga e o número de glóbulos.

Para se fazer a tripla centrifugação pela técnica de Martin e Lebeauf coloca-se numa seringa 1 $cm^3$ de citrato de sódio a 6 por cento e aspiram-se, por punção venosa, 9 $cm^3$ de sangue. Depois de bem misturados centrifugam-se durante cerca de 7 minutos. Passa-se o sobrenadante para outro tubo, que se centrifuga durante 10 minutos e em seguida, depois de se centrifugar durante 20 minutos o decantado da segunda centrifugação, observa-se o sedimento obtido, a fresco, com objectiva seca forte, ou após coloração. Número de rotações de centrífuga: 1500 por minuto.

A técnica de Máximo Prates tem a vantagem de ser menos demorada, sem que por isso dê percentagem de exames positivos muito diferente. Para a realizar junta-se 1 $cm^3$ de sangue venoso a 5 $cm^3$ de citrato de sódio a 1 por cento, colocam-se a centrifugar, a 1000-1500 rotações por minuto, durante 3 minutos, passa-se o sobrenadante para novo tubo, que se centrifuga durante 7 minutos, decanta-se e observa-se o sedimento ao microscópio. Este sedimento é mais rico em glóbulos e plaquetas do que o terceiro sedimento obtido pelo método de Martin e Lebeauf.

A primeira centrifugação é a parte mais delicada destes métodos. Antes de terminar o tempo estabelecido para a primeira centrifugação deve-se observar em que altura vai a sedimentação para se evitar que o sobrenadante chegue e ficar límpido e transparente — os melhores resultados do método obtêm-se quando no sobrenadante permanecem ainda ligeiras nuvens de glóbulos.

O sangue obtido por escarificação da lesão inicial causada pela picada da glossina ou das tripanides pode permitir também o diagnóstico precoce da doença.

Não menos útil que o exame de sangue é o exame do suco ganglionar. Desde que o indivíduo suspeito de ter doença do sono tenha reacção ganglionar, é habitualmente submetido a exame do suco em preparação a fresco — que tem a vantagem de os movimentos dos tripanossomas facilitarem a pesquisa —, ou em preparação corada, que se pode observar em qualquer altura.

Fixando-se um gânglio, geralmente das cadeias laterais do pescoço, com dois dedos, contra a pele, de modo que fique imóvel, faz-se com toda a facilidade e sem risco a punção e recolha da linfa. Estando o material esterilizado, bem seco, e fazendo-se o exame a fresco, entre lâmina e lamela, não se gasta muitas vezes mais de 10 minutos para puncionar um gânglio, recolher a linfa e observar um tripanossoma. Este exame, sujeito a variações de positividade semelhantes às do sangue, dá uma percentagem de resultados positivos muito elevada — varia entre 87,7 por cento (Broden) e 98 por cento (Dutton e Todd).

O outro exame que completa a tríade a que obrigatòriamente deve ser submetido todo o suspeito de ter doença do sono é o exame de L. C. R. Este exame, que deve ser sistemàticamente praticado, porque é o único que dá informação precisa sobre a invasão dos centros nervosos, além dos dados que fornece à terapêutica e ao prognóstico, permite ainda a pesquisa de tripanossomas. A presença de tripanossomas no líquido é, contudo, variável. Sice diz que o seu aparecimento não obedece a nenhuma regra. Os tripanossomas aparecem com certa frequência em indivíduos classificados como suspeitos e durante qualquer fase da doença, como recentemente foi posto em evidência por Neujean. É irregular o seu aparecimento no final do período nervoso — 16 por cento de casos positivos (Martin e Lebeauf) — e frequente em doentes com sintomas graves — 85 por cento de positividades segundo estes mesmos autores.

A pesquisa de tripanossomas no líquido deve ser feita no sedimento obtido por centrifugação a pequena velocidade e, se possível, em ambiente refrigerado.

Quando os exames de sangue, do suco ganglionar ou do L. C. R. não permitem revelar a presença de tripanossomas, pode-se recorrer ao exame de derrames, às inoculações a animais — pouco úteis, em especial nos casos de infecção causada pelo *Tripanosoma gambiense* — e ao exame da medula esternal. O mérito do exame da medula reside em permitir pôr, por vezes, em evidência tripanossomas quando os outros métodos não os revelam.

Pode-se ainda recorrer à hemocultura e à mielocultura. São métodos de diagnóstico muito úteis e sensíveis. Utilizando-se os meios de Brutsaert e Henrard, o meio de Weinman modificado por Henrard ou este meio após a substituição do sangue humano pelo de boi,

obtêm-se sistemàticamente culturas positivas em doentes com tripanossomas reveláveis pelos processos habituais de diagnóstico e, algumas vezes, em doentes em que os processos habituais de pesquisa não revelam a sua presença. Por este motivo, a hemocultura e a mielocultura têm valor, não só quando se tem em vista a descoberta de doentes novos em que os processos habituais de pesquisa dão resultados negativos, mas também quando se pretende averiguar a eficácia ou insucesso dum tratamento ou a duração duma infecção crónica em animais de laboratório. Em alguns doentes, porém, não se conseguem obter hemoculturas positivas. Esta perda da capacidade de cultura parece estar relacionada com a perda da capacidade de transmissão cíclica que pode ocorrer em estirpes que permanecem longo tempo no mesmo hospedeiro.

As culturas de tripanossomas são sempre muito menos ricas do que as culturas de bactérias. Não há nenhum meio artificial que permita uma multiplicação de tripanossomas semelhante à que se pode observar numa infecção intestinal bem estabelecida duma glossina. Pode-se considerar já boa cultura a que permite observar, na maior parte dos tubos semeados, rosáceas e 3 a 6 tripanossomas por campo. Mas este achado não é a regra, pois, por vezes, há culturas feitas com sangue de doentes, com gota espessa positiva, ou de doentes em que os processos habituais de pesquisa não revelam a presença do parasita, em que o desenvolvimento é muito escasso. O exame de uma só gota de cada tubo de cultura pode, nestes casos, não chegar para descobrir a existência de tripanossomas e, quando assim acontece, a tripla centrifugação do meio torna-se valiosa, visto encurtar o tempo da pesquisa.

Dos meios de cultura referidos, o que melhor resultado parece dar é o de Weinman modificado por Henrard.

Para a sua preparação distribui-se solução de gelose (3 g de gelose para 1 l de soro fisiológico), nas quantidades de 4 $cm^3$ para cada tubo, e em seguida, após esterilização feita no autoclave a 120°C durante 20 minutos, junta-se a cada tubo 2 $cm^3$ de sangue humano citratado a 1 por cento, de forma a ficar em cada tubo sangue de um só dador. O meio deve ficar líquido.

Para se fazer a hemocultura ou a mielocultura colhem-se 5 $cm^3$ de sangue venoso ou medular, para uma seringa com 1 $cm^3$ de solu-

ção de Liquoid Roche a 1 por cento, e em seguida distribuem-se em 6 tubos de cultura, nas quantidades de 1 $cm^3$ para cada tubo.

Feita a sementeira, colocam-se os tubos num armário, ao abrigo da luz, e agitam-se uma ou duas vezes por dia, durante 9 dias.

No décimo dia observam-se as culturas, fazendo-se o exame a fresco de 1 gota de cada tubo.

É necessário cuidado ao fazer-se a escolha dos dadores de sangue para o meio-base. Tanto o sangue humano como o de boi, se tiverem tripanossomas, podem falsear os resultados da cultura, visto as condições de preparação do meio-base não acarretarem a sua destruição nem impedirem o seu ulterior desenvolvimento.

Os métodos indirectos de diagnóstico — formol, gelificação, reacção de desvio de complemento, muito melhorada por Evans e Schoeners, provas de precipitação, de alergia, etc. — podem dar informações úteis, mas não permitem a caracterização exacta da doença.

# LA TRYPANOSOMIASE RÉSIDUELLE EN AFRIQUE OCCIDENTALE FRANÇAISE

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Depuis 1953, le nombre des nouveaux trypanosomés dépistés a diminué: de 6.902 en 1953, il est descendu à 5.664 puis 4.973 et remonté en 1956 à 5.334 pour arriver à 4.921 en 1957.

Pendant ce temps, les indices de contamination nouvelle et de virus en circulation, après avoir diminué régulièrement jusqu'en 1956, montraient une légère tendance à remonter en 1957. Parti de 0,12 en 1953, l'I.C.N. passait à 0,10, 0,098, 0,090 et enfin 0,096. Pendant ce temps l'I.C.T. donnait: 0,77, 0,71, 0,66, 0,39 et 0,46 en 1957. L'I.V.C. de 0,10, à 0,08, 0,07 et 0,07, 0,09.

La discordance entre la courbe du chiffre brut des nouveaux trypanosomés et celles des indices s'explique par l'absentéïsme de plus en plus important aux séances de prospection. L'indice de présence n'a pas cessé de diminuer et est arrivé à 67 pour cent en moyenne pour l'A.O.F. en 1957.

Les 2/3 seulement des nouveaux trypanosomés ont été dépistés par l'ensemble du système de contrôle: équipes mobiles, postes-filtres, et contrôle de la main-d'œuvre saisonnière au départ. Ce dernier service voit son efficacité diminuer chaque année, car les travailleurs groupés sont de plus en plus rares. Ils voyagent isolément, évitent ainsi les examens médicaux et vaccinations avant de quitter leur territoire d'origine. Le dernier tiers est formé par les gens qui viennent se faire examiner dans les formations sanitaires du service d'Hygiène mobile. Ils sont presque tous en deuxième période et l'atteinte de l'axe cérébro-spinal est souvent sévère. Nous avons malgré tout

un pourcentage de 37 pour cent de malades en période lymphatico-sanguine parmi ces 4.921 nouveaux trypanosomés en 1957.

L'absentéïsme est dû au fait que la maladie du sommeil étant devenue aux yeux des Africains une endémie mineure, les populations ont une impression, hélas bien fausse, de sécurité et presque partout ne se dérangent plus pour venir aux rassemblements de la prospection ou fuient la seringue de l'infirmier qui vient faire des injections préventives de lomidine.

Mais si la trypanosomiase n'est plus aussi répandue qu'autrefois dans l'ensemble de l'Afrique Noire, il n'en reste pas moins des endroits le long des principaux cours d'eau ou des grandes routes où elle sévit de façon endémique et doit être surveillée de très près pour éviter son extension.

En détaillant sa répartition actuelle, nous trouvons un certain nombre de foyers en activité.

Un foyer assez important s'est développé en 1957 dans la région de M'Bour, petit port de pêche situé à 80 km au Sud de Dakar où 112 nouveaux trypanosomés, dont les 2/3 en période lymphatico-sanguine, ont été dépistés dans le canton dont M'Bour est le chef-lieu. Ce foyer n'est pas encore éteint à cause de l'incompréhension des habitants qui répugnent à venir aux examens systématiques et à se faire protéger contre le trypanosome.

## LE FOYER DES FRONTIÈRES DE L'A.O.F.

Le foyer qui s'étendait le long des frontières avec la Guinée et la Côte d'Ivoire, d'une part, le Sierra Leone et le Libéria, de l'autre, est en voie d'extinction, y compris la petite portion de territoire guinéen qui s'enfonce en coin dans le Sierra Leone près du Libéria, et la région du Cavally et du Sassandra, avec son affluent de Nzo, en Côte d'Ivoire. Le Cavally forme frontière avec le Libéria et ses rives sont restées longtemps très infestées.

Ces dernières années, à la faveur de l'indifférence et de l'apathie manifestée par la population en face de lui, le foyer avait débordé vers l'est et pénétré dans le bassin de Sassandra. Il a pu être maîtrisé en deux ans et sur tout le pourtour du Libéria et du Sierra Leone on n'a plus trouvé que 782 nouveaux malades, dont le 1/5 seulement

dans la phase lymphatico-sanguine. Ceci est dû aux prospections constantes et aux lomidinisations préventives faites dans ces régions depuis 1948.

Mais le danger paraissant s'éloigner, les populations redeviennent réticentes et nous ne sommes pas à l'abri d'un retour offensif comme dans la région de M'Bour.

Le long de la frontière du Ghana, à la hauteur d'Abengourou, Adzopé, Agboville, en face du pays des Ashantis, un foyer important et très actif s'est développé ces trois dernières années.

Dans ces trois cercles, on a trouvé, en 1957, 849 nouveaux trypanosomés, dont 252 à la prospection avec un pourcentage de 67 pour cent en première période. Le faible rendement des équipes de prospection, opérant de la façon habituelle d'après les recensements administratifs tenus à jour chaque année, est dû au fait que les nouveaux trypanosomés sont, pour la plupart, des étrangers non recensés vivant en pleine brousse et échappant à tout contrôle médical, à toute prospection, population flottante de travailleurs descendant de Haute-Volta et du Soudan, de commerçants qui circulent constamment à travers la frontière du Ghana.

Les commerçants sont également dépistés dans les postes-filtres. Pour pouvoir atteindre les travailleurs étrangers dans les meilleures conditions, c'est-à-dire le plus tôt possible, avant qu'ils aient eu le temps de disséminer leurs trypanosomes, il a été créé un système de prospection «en marguerite» appelé *barrage mobile*.

Une équipe de microscopistes s'installe à un carrefour de routes à proximité d'un groupe de campements, et des infirmiers vont les examiner et ramènent les suspects.

Par ce procédé, 81 nouveaux malades ont été trouvés, dont 47, soit 58 pour cent, en période lymphatico-sanguine.

Le cercle d'Abengourou est le plus infesté avec 600 nouveaux trypanosomés en 1957 (470 en 1956), puis vient le cercle d'Agboville avec 121 nouveaux malades (64 en 1956), dont 45 pour cent en première période avec un index moyen de contamination nouvelle de 1,16 pour cent.

Ceci s'explique par le grand nombre de travailleurs étrangers qui viennent dans les plantations.

Un autre foyer se trouve au Nord-Ouest du Dahomey, dans la boucle de la Pendjari ou Oti, et entre cette rivière et la frontière

avec le Togo et la Haute-Volta. Ce foyer, résidu de l'endémie ancienne, est très difficilement réductible à cause de l'indocilité des Berbas, qui se refusent à venir à la prospection et encore moins à la lomidinisation. Pourtant celle-ci a pu être faite en partie en 1956, où une montée du nombre de nouveaux malades a rendu les gens plus dociles et leur nombre a diminué.

Il était passé à 183 au lieu de 106 à 116 les années précédentes. En 1957 il est retombé à 105. Sur ce nombre il y en avait 80, dont 53, soit 66 pour cent, en période lymphatico-sanguine dans les 6 cantons en bordure du Togo, domaine des Berbas, particulièrement peu ouverts à la médecine préventive. Ces gens se contaminent le long de la rivière et aussi pendant leurs voyages dans les pays voisins: Togo et Ghana.

*À l'intérieur du pays,* le long du Niger, le foyer de Bamako, qui n'a jamais pu être réduit, s'étend depuis 2 ans, par suite de la mauvaise volonté des habitants à se présenter aux prospections et aux séances de lomidinisation préventive.

Autrefois limité autour de Bamako, il a gagné en aval jusqu'aux environs de Niamina, chef-lieu de canton à mi-chemin entre Koulikoro et Ségou, et, en amont, est remonté jusqu'en Guinée le long du Niger et de ses affluents: le Sankarani à droite, le Tinkisso à gauche, dans les Cercles de Siguiri et de Kankan, région de savane à l'est-nord-est de la Guinée. Il s'élargit en remontant le fleuve; limité à une bande de quelques kilomètres de large jusqu'au sud-ouest de Bamako, il s'étale dans les subdivisions riveraines en amont de cette ville, pour gagner en Guinée plus de la moitié des cercles de Siguiri et de Kankan. La partie la plus active est à Bamako et ses environs immédiats. En 1956, 184 nouveaux trypanosomés, dont 5 européens et 1 libanais, ont été dépistés dans la ville et ses environs jusqu'à Kati. Plus de 60 pour cent étaient en première période. Sur 49.048 personnes visitées on n'a pu faire que 1.288 lomidinisations, dont 47 sur des Européens.

En 1957, à Bamako, on a pu examiner 101.623 individus et dépister 81 nouveaux trypanosomés, dont 2 européens. Les deux tiers de ces malades étaient en première période.

Ces résultats ont été obtenus grâce à l'aide de la municipalité et des autorités locales, qui ont organisé une propagande préliminaire

à notre action par voie de presse, affiches, émissions de radio et haut-parleurs. Des travaux de prophylaxie agronomique ont été poursuivis le long du Niger et de ses affluents autour de Bamako, la Farako et l'Oyanko, toujours avec l'aide de la municipalité. On a lomidinisé 1.466 personnes.

Dans l'ensemble du foyer soudanais on a trouvé, en 1957, 813 nouveaux trypanosomés, dont 60 pour cent en première période, avec un indice de contamination nouvelle moyen de 0,72 pour cent dans les cantons visités.

Enfin, au Soudan toujours, le long de la Bagoé, affluent du Niger, il reste un foyer à peu près permanent qui s'étend sur 9 cantons. Après lomidinisation, on n'a plus trouvé, dans 4 de ces cantons prospectés en 1957, que 95 nouveaux trypanosomés, dont 59 en première période, sur 41.547 personnes visitées.

Dans la partie Est du Soudan et dans la région de savane de la Haute-Volta, de nombreux petits foyers erratiques se rencontrent tous les ans. Ce sont des quartiers, des villages, parfois un groupe de villages contaminés par des voyageurs, soit étrangers venant colporter trypanosomes et marchandises diverses, soit travailleurs revenant des plantations ou mines du Ghana et de Côte d'Ivoire. Ce n'est jamais bien grave dans ces régions où le souvenir de l'endémo-épidémie sommeilleuse n'est pas encore éteint et où les gens viennent à peu près régulièrement aux prospections en temps ordinaire, avec impressement quand ils sentent le danger menaçant. On voit alors, chose qu'on ne rencontre plus ailleurs, jusqu'à 95 pour cent de la population qui vient se faire examiner et traiter. L'extinction rapide du foyer montre que la lomidine a toujours la même action préventive, d'autant plus efficace que le nombre de sujets protégés est plus grand.

Partout ailleurs, il y a peu de malades nouveaux. Le long de la Côte cependant, surtout en Côte d'Ivoire, on trouve encore de nouveaux malades disséminés dans les plantations et il convient de les surveiller. De même en Guinée.

Le Bas-Dahomey, le Haut-Sénégal et l'Ouest et le Nord du Soudan sont de moins en moins touchés et on ne trouve dans ces vastes territoires que de très rares trypanosomés étrangers au pays. Il en est de même au Niger, où dans la région de Niamey, autrefois assez touchée pour avoir nécessité la création d'un secteur spécial, on ne trouve plus qu'un ou deux malades par an, commerçants qui

se sont contaminés le plus souvent au Ghana. La trypanosomiase devient dans cette région du ressort de la médecine individuelle.

En somme, la trypanosomiase reste encore dangereuse en A.O.F. dans les régions où il y a des déplacements de population. C'est une maladie qui a perdu beaucoup de son importance, puisque, pendant ces dernières années, nous n'avons trouvé qu'un indice de contamination très faible, inférieur à 0,1 pour cent malgré le relèvement de 1957. Elle reste néanmoins encore menaçante, comme le prouvent les flambées de M'Bour et de Bamako et l'extension du foyer soudanais.

## RÉSUMÉ

La trypanosomiase humaine en A.O.F. continue de constituer une endémie menaçante.

Si elle a pratiquement disparu en un territoire — Niger —, si elle est dans l'ensemble ramenée à un taux rassurant dans la plupart des autres territoires, elle ne peut absolument pas être considérée à la légère comme une endémie mineure ou en voie d'extinction et continue d'imposer une surveillance aussi constante que draconienne dans toute l'étendue des zones à glossines vectrices des sept territoires intéressés.

Le cliché bien connu reste malheureusement vrai: «L'incendie semble éteint mais les braises couvent sous la cendre...»

La trypanosomiase demeure menaçante dans les foyers suivants:

*Le long de la Petite Côte* de la presqu'île du Cap Vert au sud de Dakar, à côté de M'Bour, dans le canton de Nianning, où 112 nouveaux trypanosomés ont été dépistés cette année avec un I.C.N. de 0,71 pour cent.

*Le long des frontières: du Sierra Leone* et *du Libéria,* qu'elles coiffent depuis la région de Mamou, en Guinée, jusqu'à celle de Danané et Duékoué, en Côte d'Ivoire, le long des deux fleuves côtiers, le Cavally et le Sassandra, avec son affluent le Nzo, dans leur cours moyen (782 nouveaux trypanosomés en 1957); *du Ghana,* dans les régions d'Abengourou, Agboville, Adzopé (849 nouveaux trypanosomés en 1957, dont 600 dans le cercle d'Abengourou, avec 1,16 pour cent d'indice de contamination nouvelle); *du Togo,* dans le cours supérieur du Pendajari au Dahomey (105 nouveaux trypanosomés en 1957, dont 66 pour cent en première période).

*Le long du cours supérieur du Niger,* depuis les cercles de Siguiri et Kankan, en Guinée, jusqu'à Niamina, à mi-chemin entre Bamako et Ségou (813 nouveaux trypanosomés en 1957, avec 0,72 pour cent d'indice de contamination nouvelle).

Plus à l'Est, le long de la Bagoé, autre affluent du Niger, un foyer existe aussi où on a trouvé, en 1957, 95 nouveaux trypanosomés, dont 59 en première période, dans 4 cantons riverains prospectés.

Les territoires à l'Est du Soudan et la Haut-Volta, dans la région des savanes, sont la siège de petits foyers erratiques autour de cas importés dans le voisinage des cours d'eau ou des mares à glossines et rapidement éteints par la stérilisation des malades et la lomidinisation des individus sains.

Les foyers encore actifs sont entretenus et souvent provoqués par l'inconscience des populations, qui, rebelles à toute discipline et inconscientes du danger, estiment avoir le droit de se soustraire aux prescriptions d'hygiène et contaminent ainsi gravement leurs voisins.

# INVESTIGAÇÕES SOBRE O BLEFAROPLASTO (KINETOPLASTO) DOS TRIPANOSSOMAS

HEINZ MÜHLPFORDT [1]

O blefaroplasto, também chamado *kinetoplasto*, é a característica distintiva mais importante da família *Trypanosomidae*. Sobre a sua função e a sua importância existem várias hipóteses; todas elas requerem ainda investigações experimentais.

Nesta conferência relatam-se as observações feitas durante a criação de tripanossomas sem blefaroplasto por meio da tripaflavina. O blefaroplasto, em comparação com o núcleo muito pequeno, é demonstrável com os corantes comuns, como Giemsa e outros. Mas para investigações experimentais eles não são satisfatórios, porque o plasma reage com a produção de volutina. Às vezes estas estruturas podem ser confundidas com o blefaroplasto. Como o blefaroplasto, da mesma maneira que o núcleo, tem uma reacção nuclear positiva, a reacção Feulgen poderia ser utilizada para a demonstração específica. Inicialmente pratiquei esta técnica, mas obtive melhores resultados com a coloração com ácido clorídrico-Giemsa, também específica para a cromatina. Os núcleos, os blefaroplastos e os flagelos apresentam-se em vermelho-escuro e o tripanossoma em vermelho-claro. Esta coloração dá bons resultados, mas em alguns casos não se conseguiu diferenciar com segurança absoluta a presença ou a ausência do blefaroplasto. Por isso desenvolvi a seguinte técnica:

Corei os tripanossomas com ácido clorídrico-Giemsa e utilizei, em vez da luz normal, a óptica de contraste de fase. À primeira vista este processo parece absurdo, mas o resultado é magnífico. O núcleo e o blefaroplasto aparecem em azul e em todos os casos se pode dis-

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tinguir, clara e indubitàvelmente, a presença ou ausência do blefaroplasto.

Investigou-se comparativamente a acção da tripaflavina sobre o blefaroplasto das seguintes espécies: *T. equiperdum, T. evansi, T. gambiense, T. brucei, T. congolense, T. lewisi* e *T. cruzi*. Para permitir uma comparação dos tripanossomas investigados, realizaram-se as investigações sempre com a mesma técnica, com a mesma diluição da tripaflavina de 1:2500 e a mesma dosagem referida ao peso dos animais em investigação.

De início verifiquei, pelo mesmo método, qual o tempo necessário para criar estirpes sem blefaroplasto.

No caso de *T. equiperdum* o número dos tripanossomas sem blefaroplasto aumenta muito ràpidamente e já em 54 dias a estirpe de *T. equiperdum* se apresentou sem blefaroplasto algum. Foram praticadas só 3 passagens. *T. evansi* comportou-se de maneira muito semelhante. Esta estirpe precisou de 5 passagens em 81 dias para se tornar absolutamente ablefaroplástica. Os blefaroplastos da estirpe de *T. brucei* foram completamente eliminados em 121 dias, o que corresponde a 11 passagens, e os de *T. gambiense* em 122 dias, ou sejam 19 passagens. No caso de *T. congolense* só consegui obter com a minha técnica um máximo de 64 por cento de tripanossomas sem blefaroplasto. Foi-me também impossível criar uma estirpe de *T. lewisi* sem blefaroplasto. O máximo de tripanossomas ablefaroplásticos alcançado neste caso foi de 55 por cento. Os blefaroplastos de *T. cruzi* não mostraram reacção alguma e jamais pude observar um único tripanossoma sem blefaroplasto.

Não há dúvida alguma de que os blefaroplastos das espécies estudadas possam reagir de maneira diferente, caso se aplique outro método, uma dosagem diferente e, naturalmente, sob a influência de outras drogas. Mas o essencial destas minhas investigações foi observar o efeito da tripaflavina sobre várias espécies, aplicando a mesma dosagem, e a mesma técnica, para desenvolver pontos de vista gerais referentes ao comportamento do blefaroplasto.

Como reagem os blefaroplastos à acção da tripaflavina e de que maneira se formaram tripanossomas sem blefaroplasto?

Nesta questão defrontam-se várias opiniões. Uma perturbação no mecanismo de divisão, sem qualquer outro efeito, é a solução mais simples, aceite pela maioria. Segundo a opinião de Laveran, Roudsky

e Steffan, o blefaroplasto é destruído no próprio local. Kudicke e Gonder observaram uma migração em direcção ao núcleo. O emprego da coloração ácido clorídrico-Giemsa em combinação com o microscópio de contraste de fase permitiu estudar mais exactamente o comportamento do blefaroplasto. O blefaroplasto perde a sua posição na vizinhança do corpo basal, migra em direcção ao núcleo, passa ao lado dele e é totalmente expelido na parte anterior. Observa-se nìtidamente este comportamento em *T. lewisi* e *T. congolense*. São estas as espécies em que o blefaroplasto se mostrou, nas minhas experiências, mais resistente ao tratamento com tripaflavina. Nestas espécies nem sempre foi eliminado totalmente, mas fraccionou-se às vezes antes de alcançar o núcleo, sendo os fragmentos absorvidos. Esta fragmentação é rara nos casos de *T. lewisi* e *T. congolense*, mas é regra nas outras espécies, tais como *T. equiperdum*, *T. evansi*, *T. brucei* e *T. gambiense*. Nos últimos só excepcionalmente o blefaroplasto deslocado atinge a parte anterior do tripanossoma.

Uma consideração comparativa dos resultados permite as seguintes conclusões:

1) No caso de *T. equiperdum* e *T. evansi* o número dos tripanossomas sem blefaroplasto aumenta consideràvelmente logo após o primeiro tratamento e depois de 10 dias já se vêem 75 a 90 por cento sem esta estrutura. Esta observação e o facto de nas espécies mencionadas aparecerem com facilidade variedades sem blefaroplasto algum estão de acordo com as investigações de Wenyon, Hoare e Tobie, que falam de variedades sem blefaroplasto de *T. evansi* e *T. equiperdum* em condições naturais e sem qualquer tratamento. Este comportamento dá a impressão de que se trata, neste caso, dum blefaroplasto muito lábil.

2) Qualquer espécie, uma vez completamente sem blefaroplasto, nunca mais aparece com esta organela, mesmo depois de se suspender o tratamento.

3) Com a minha técnica não pude criar em *T. congolense* uma variedade sem blefaroplasto. Em muitas espécies existe, sob condições naturais, uma pequena percentagem de indivíduos ablefaroplásticos. Segundo as investigações de Jirovec, a percentagem dos tripanossomas sem blefaroplasto é muito pequena no caso de *T. congolense*. E na maioria as estirpes desta espécie carecem completamente de tais formas. Esta observação, em conjunto com os meus

resultados, é um argumento a favor da tese de que se trata, nesta forma, dum blefaroplasto mais resistente ao tratamento e a outras influências até agora desconhecidas.

4) Segundo a opinião geral, os tripanossomas sem blefaroplasto formam-se em consequência de divisões perturbadas. Sendo exacto este ponto de vista, não devia haver um efeito da tripaflavina nas espécies que não se multiplicam no sangue. *T. cruzi* é uma dessas espécies. As minhas observações confirmam esta opinião, porque nenhum indivíduo de *T. cruzi* perdeu o blefaroplasto. Em *T. lewisi,* espécie em que podemos distinguir uma fase curta de multiplicação e uma sem divisão alguma, deveria ser muito mais fácil esclarecer esta dúvida. Tratei ratas esplenectomizadas com tripaflavina na fase da multiplicação, sendo o resultado máximo 55 por cento de formas sem blefaroplasto. Como na outra fase não havia divisões, não foi visível efeito algum. Estes resultados demonstram incontestàvelmente que os indivíduos sem blefaroplasto só se formam durante a multiplicação dos tripanossomas.

5) Nas espécies investigadas, com excepção de *T. cruzi,* o blefaroplasto perde a sua posição na vizinhança do corpo basal. Deslocado do seu lugar, o blefaroplasto migra para a parte anterior do tripanossoma. O grau da sua resistência e, talvez, o estado fisiológico em que se encontra no momento da acção da tripaflavina decidem se o blefaroplasto já se fragmentará e será absorvido durante a migração, ou se será eliminado totalmente na parte anterior. Não foi possível esclarecer se esta migração é activa ou passiva e causada pelo movimento dos tripanossomas, porque é difícil investigar experimentalmente este fenómeno.

Finalmente, quero relatar os resultados de experiências com *T. gambiense*. No momento em que 63 por cento dos tripanossomas não mostravam mais blefaroplasto suspendeu-se o tratamento com tripaflavina, para observar como se altera a relação de tripanossomas com e sem blefaroplasto. Inocularam-se camundongos e ratos. Os tripanossomas com blefaroplasto suplantaram completamente os outros e em 25 dias a estirpe com blefaroplasto era normal em camundongos. Em ratos esta relação de tripanossomas comportou-se de modo semelhante. Havia só uma diferença: a estirpe normalizou-se já em 7 dias. Estas observações confirmam os resultados obtidos por outros. Neste tipo de experiências não se pode excluir que os tripanossomas sem

blefaroplasto tenham produzido, pela tripaflavina, um defeito tal que a multiplicação não seja tão rápida como nos normais. Para anular esta objecção misturei uma estirpe normal e variedade ablefaroplástica correspondente, mantida há já um ano em camundongos, numa relação de 52 para 48. Também nesta experiência os tripanossomas com blefaroplasto suplantaram completamente os outros. Em camundongos predominam, após 16 dias, e em ratos após 8 dias, os tripanossomas normais, de tal maneira que não os podemos distinguir mais de uma estirpe normal. Esta predominância dos tripanossomas sem blefaroplasto não tem, portanto, nada que ver com um dano produzido pela tripaflavina. Ainda não se sabe que factores intervêm, requerendo este fenómeno investigações ulteriores.

## BIBLIOGRAFIA


GONDER, R. — «Experimentelle Studien mit Trypanosomen und Spironemen (Spirochäten)», *Z. Immunforsch. u. exper Therapie,* **15**: 257-292, 1912.

HOARE, C. A. — «Morphological and Taxonomic Studies on Mammalian Trypanosomes. V. The Diagnostic Value of the Kinetoplast», *Trans. Roy. Soc. Trop. Med. Hyg.,* **32**: 333-342, 1938-1939.

HOARE, C. A. — «The Loss of the Kinetoplast in Trypanosomes, with Special References to *Trypanosoma evansi*», *J. Protozool.,* **1**: 28-33, 1954.

HOARE, C. A., e BENNETT, S. C. J. — «Morphological and Taxonomic Studies in Mammalian Trypanosomes. III. Spontaneous occurrence of Strains of *T. evansi* Devoid of the Kinetonucleus», *Parasitology,* **29**: 43-56, 1937.

HOARE, C. A., e BENNETT, S. C. J. — «Morphological and Taxonomic Studies on Mammalian Trypanosomes. VI. Further Observations on the Absence of the Kinetoplast in *T. evansi*», *Parasitology,* **30**: 529-542, 1938.

JIROVEC, O. — «Über das Vorkommen von blepharoplastlosen Trypanosomen in normalen Stämmen», *Zbl. Bakt. I. Orig.,* **121**: 55-58, 1931.

KUDICKE, R. — «Die Wirkung orthochinoider Substanzen auf Rattentrypanosomen», *Zbl. Bakt. I. Orig.,* **59**: 182-185, 1911.

LAVERAN, A., e ROUDSKY, D. — «Au sujet de l'action de l'acridine (diphenylmethane) sur *Trypanosoma lewisi* e *T. duttoni*», *C. R. hebd. Soc. Biol.,* **73**: 172-175, 1912.

STEFFAN, P. — «Morphologische Untersuchungen über die Wirkung verschiedener Heilmittel auf Trypanosomen», *Z. Hyg.,* **96**: 263-287, 1922.

TOBIE, E. J. — «Loss of the kinetoplast in a strain of *Trypanosoma equiperdum*», *Trans. Amer. Microscop. Soc.,* **70**: 251-254, 1951.

WENYON, C. M. — «The Loss of the Parabasal Body in Trypanosomes», *Trans. Roy. Soc. Trop. Med. Hyg.,* **22**: 85-87, 1928-1929.

# THE THIAMINE CONTENT IN LIVER, SPLEEN, KIDNEY AND BLOOD OF RATS WITH INFECTIONS OF *TRYPANOSOMA RHODESIENSE*

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Little is known of the growth requirements of the African trypanosomes. As yet the blood stream form of these organisms has not been cultivated, and although the insect forms may be grown on fluid, semi-solid or diphasic medium, they rapidly lose their infectivity. Recently it was reported that the infectivity of *Trypanosoma rhodesiense* may be restored in the presence of trehalose.

On the other hand, certain of the trypanosomidae of lower animals and insects have been cultivated on a chemically defined medium. Among the essential nutrients required by these organisms has been thiamine. This was first determined for *Strigomonas oncopelti,* an hemipteran parasite, which may be grown on peptone water alone. It has further been shown that thiamine is an essential part of the peptone and may not be replaced by either the thiazole or the pyrimidine parts of the molecule separately or together. *S. fasciculata* as well as *S. culicidarum* and *Leishmania tarentolae* are also incapable of synthesizing thiamine.

Studies on the pathogens and some related organisms have been chiefly concerned with the effects of changes in host nutrition on the course of infection. A prolonged parasitemia and higher multiplication rate for *Trypanosoma lewisi* results in rats on either a pantothenate or biotin deficient diet. Doves, ordinarily refractory to infection with *Trypanosoma brucei,* may be infected after they have been exposed

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to a vitamin B complex deficient diet. The above cited indicate a loss of resistance due to diet deficiency. On the other hand, it has been shown that resistance in rats to *Trypanosoma equiperdum* was enhanced after they had been placed on a vitamin B deficient diet. The trypanosomes appeared at the same time and multiplied at the same rate as in the controls, but infected deficient animals lived 2-6 days longer. Indeed, the same effect could be achieved by placing the animals on a thiamine deficient diet.

The present report is an attempt to define a role for thiamine in rats with infections of *Trypanosoma rhodesiense*.

*Thiamine concentration*[1] *in rats with infections of* Trypanosoma rhodesiense

| Rats | Age (days) | Weight (grams) | Parasites x $10^5$ mm$^3$ | RBC x $10^5$ mm$^3$ | µg thiamine per 100 ml | µg thiamine per gram[2] | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Blood | | Liver | Spleen | Kidney |
| 1, 2 . . . . | 31 | 60 | 0.9 | 60.0 | 26.3 | 17.3 | 1.8 | 10.0 |
| 3, 4 . . . . | 32 | 58 | 2.0 | 61.0 | 37.5 | 25.5 | 1.1 | 9.5 |
| 5, 6 . . . . | 33 | 51 | 7.0 | 64.0 | 72.6 | 26.3 | 1.3 | 5.6 |
| 8. . . . . | 34 | 38 | 14.0 | 43.0 | 130.0 | 30.0 | 3.7 | 3.0 |
| 4 controls . . | 31–34 | 50 | — | 69.0 | 35.0 | 15.2 | 3.1 | 8.0 |
| 23, 24 . . . | 113 | 224 | 0.2 | 90.0 | 25.0 | 10.9 | 1.2 | 17.6 |
| 25, 26 . . . | 114 | 232 | 1.7 | 95.2 | 31.3 | 10.5 | 1.6 | 18.8 |
| 27, 28 . . . | 115 | 232 | 6.1 | 78.3 | 65.0 | 17.3 | 2.4 | 17.3 |
| 30, 36 . . . | 116 | 217 | 9.2 | 66.0 | 93.7 | 13.1 | 2.8 | 4.1 |
| 6 controls . . | 113–116 | 222 | — | 88.3 | 37.3 | 11.6 | 1.5 | 8.5 |

1 All values are averages for animals listed in «Rats» column.
2 Wet weight.

The thiamine content of liver, spleen, kidney and blood of rats with infections of *T. rhodesiense* has been determined. Two groups of 10 rats (Sprague-Dawley females) each were infected with $1.2 \times 10^9$ parasites per kilogram and two groups of 6 animals were kept as controls. At infection one control and one experimental group were 31 days old, with weights ranging from 42-60 g. The other two groups were 113 days old and weighed 208-245 g. Parasites

could be found in the peripheral blood immediately after infection. In the two infected groups, 2 animals were sacrificed each day starting on the first day after infection. All animals were bled by cardiac puncture. The organs were then removed, weighed, frozen in a dry-ice alcohol mixture and stored in a deep freeze.

For assay, organ samples were thawed, ground in tissue homogenizers with acetate buffer (pH 4.5), and digested under benzene with papain and takadiastase for 24 hours at 37°C. Tissues so treated were then properly diluted and added to the assay medium. Tubes were then inoculated with *Lactobacillus fermentum,* incubated for 16-18 hours at 37°C and compared with a standard set turbidimetrically in a Klett-Summerson colorimeter.

In the blood, anemia developed as parasitemia increased over the 4 day course of infection. With the rise in parasitemia there was a concomitant increase in the amount of thiamine found in the whole blood. In the younger animals, the thiamine values rose from about 42.5 $\mu$g/100 ml to 130, while in the older rats it increased from 25 to 100 $\mu$g/100 ml. We have been unable, as yet, to explain this rise in thiamine in the blood.

Almost immediately after infection the ratio of liver to body weight was increased slightly in both the young and the older infected groups. In the livers of the young animals thiamine concentration showed an increase by the second day of infection and in some cases animals sacrificed later showed an almost doubled concentration.

The spleens of infected animals increased from 3-5 times their normal size. There appeared to be a slight drop in thiamine concentration in the young animals and no change in the older rats.

The kidney showed no change in organ to body weight ratio during the infection. In the older group an increased thiamine concentration was apparent 24 hours after infection. Terminally, or 4 days post infection, there was a drop in both young and old rats to almost 1/3 that found in normal tissue.

Thus, livers of young animals showed an increase in thiamine concentration, spleens showed a decrease which may not be significant, and the kidney no change until the terminal stages of the infection. In the older animals, there was no change in either the liver or the spleen. The kidney showed a marked increase initially and a marked drop terminally.

With the present data we cannot as yet ascribe a role to thiamine for the trypanosome. In view of the thiamine requirement in *Strigomonas,* it is unlikely that a more fastidious parasite such as *T. rhodesiense* might synthesize this vitamin. Indeed one might even wonder about the presence of thiamine in this organism. The chief end product of aerobic fermentation in the African trypanosomes is pyruvic acid, an alpha keto acid. How then do these organisms use thiamine which is known to be a portion of cocarboxylase, the coenzyme of an alpha keto decarboxylase? Further, if the trypanosomes required large amounts of thiamine, we might expect an appreciable drop in concentration in the various organs studied. Such has been the case for coenzyme A in the livers of ducks and rats infected with malaria, for ascorbic acid in the livers of guinea pigs with *Trypanosoma brucei,* and for rat livers, kidneys and adrenals with infections of *Trypanosoma hippicum.*

Therefore, the changes which we have seen may simply reflect a derangement of the host metabolism. In view of the known tremendous glucose utilization by the parasite and the large amounts of pyruvate which must be formed, we can assume that the increase of thiamine in the blood reflects an attempt by the host to utilize and/or detoxify the metabolic products of the trypanosome. The early increased concentration of thiamine in the kidney may indicate reabsorption of the vitamin and the terminal drop a breakdown of this mechanism. This is in agreement with the observation of others that pyruvate is handled by the infected host almost as rapidly as it is formed, but is not consonant with the report that thiamine deficient rats show an increased survival time with *T. equiperdum* infections. In the latter case, we would have expected increased susceptibility.

# NORMAL RAT SERUM AS A GROWTH FACTOR FOR *TRYPANOSOMA LEWISI*

DAVID RICHARD LINCICOME

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and
Howard University, Washington, D.C., U.S.A.

One of the pressing present-day problems confronting the experimental parasitologist is concerned with the cellular metabolic exchanges that accompany parasitism. Experimental systems to study these phenomena have been difficult to obtain since so many organisms live in the alimentary tract of their sheltering hosts, where it is impossible to control the environment by conventional techniques.

*Trypanosoma lewisi,* a highly host-specific parasite of the rat, may be employed as a tool for such studies with profit since it may live in the blood stream in the absence of accompanying flora or fauna. The work of Desowitz and Watson (1951) in establishing *Trypanosoma vivax* in rats has suggested the possibility of employing *T. lewisi* in heterologous hosts as a useful biologic system for analysis of many chemophysiologic problems.

The following account is concerned with results of a study of *Trypanosoma lewisi* in heterologous mouse hosts.

After intraperitoneal inoculation into well-fed albino mice, *T. lewisi* may be detected in peripheral circulating tail blood for approximately 24 hours, after which they cannot be recovered in saline smears (see Fig. 1). Even after caloric restriction of the mouse's diet survival time is still not prolonged beyond a day (Lincicome, 1955, 1958) as judged by fresh, wet preparations of peripheral tail blood.

The administration of 1 ml of normal rat serum per day per mouse after introduction of the trypanosomes is associated with increased survival time of the parasites up to 9 days if the diet of the host is normal. There does not appear to be any marked growth in numbers of trypanosomes in these hosts. The population level in peripheral blood is comparable to that originally introduced. Survival

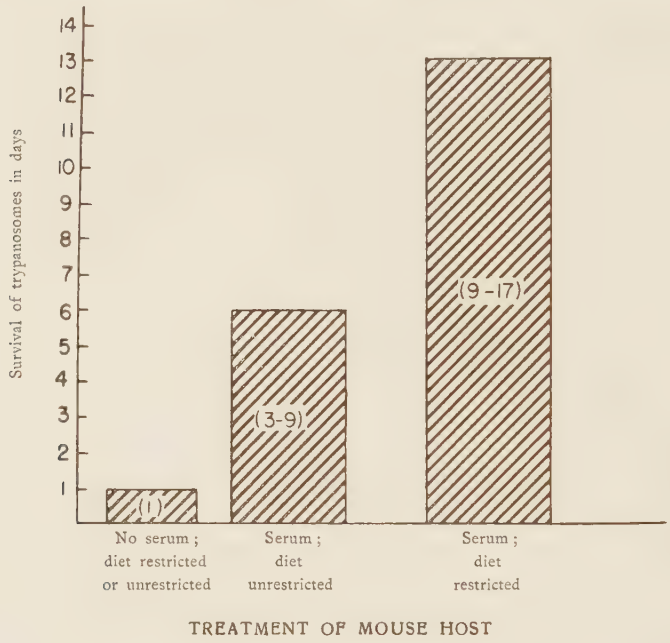


Fig. 1 — Survival of *Trypanosoma lewisi* in mice with and without supplements of rat serum and calorically restricted diets

with limited reproductive potential therefore seems to describe their existence adequately.

If, however, the caloric intake of the mouse is sharply reduced by initial starvation followed by an allowance of 1 g/day/mouse, not only is the parasite's survival extended up to 17 days (see Fig. 1), but large numbers develop and circulate in tail blood (see Fig. 2). The reproductive potential here is obviously great. Death of the host frequently occurs at the height of the parasitemia (Lincicome, 1958).

Trypanosome growth in heterologous mouse hosts, maintained on calorically restricted diets and supplemented with normal rat serum daily, has been sufficiently predictable so that serial subpassage to

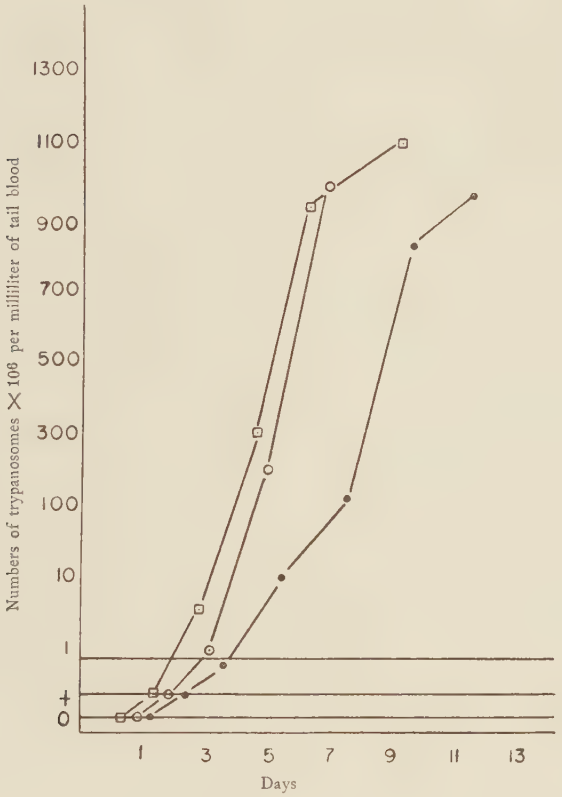


Fig. 2 — Growth of *Trypanosoma lewisi* in starved albino mice receiving rat serum supplements and varying levels of parasites in the inoculum:

● $0.145 \times 10^6$
○ $0.725 \times 10^6$
□ $1.45 \times 10^6$

*(From Lincicome, 1958)*

other mice has been attempted. This parasite has been passed from mouse to mouse more than 275 times without resort to passage in the usual host animal. Supply of normal rat serum still remains as a requisite for continued growth.

At the 75th passage in starved mice it was found that it was no longer necessary to reduce the host's caloric intake to facilitate development of the parasite. This line of trypanosomes has been continued in unstarved mice hosts now for 197 passages without intercalation of the rat host, but the organism still requires the assistance of daily normal rat serum supplements.

The development of *Trypanosoma lewisi* in the heterologous mouse host depends upon some factor in normal rat serum because without this the parasite fails to survive beyond a few hours. Passage in the mouse does not impair its ability to grow in the usual host; therefore, there is apparently little or no gross metabolic change in the parasite.

The mechanism by which rat serum facilitates the growth of this trypanosome in an unnatural host has been considered to be due either to the interference with the operation of the defensive machinery of the mouse or to the supply of a nutritional factor needed by the parasite. It is also possible that the rat serum may be wholly protective as Pollack (1947) and Davis and Dubos (1946) have observed in cultures of the pertussis organism and the tubercle bacillus.

The first hypothesis relating to blockade of host immunity reactions appears not to offer an adequate explanation of the phenomenon. Administration of sodium salicylate to mice does not alter the environment within the mouse sufficient to permit the development of the trypanosome in the absence of rat serum. Administration of serum for several days prior to inoculation of parasite has not altered the subsequent growth of these organisms. Tissue blockade of the mouse with thorotrast, colloided carbon or other comparable substances that also impair the function of the reticulo-endothelial system has not been undertaken nor have blood levels of properdin been lowered by use of zymogen. It is believed that inhibition of reticulo-endothelial activity does not play a significant role in this phenomenon.

The evidence, on the other hand, suggests the view that rat serum possesses a specific factor that is required by *Trypanosoma lewisi*. This has been adopted as a working hypothesis.

Whatever factor(s) that may be possessed by normal rat serum is not shared by the sera of man, dog, rabbit, chicken, horse, pig and possibly hamster serum, but at present this has not been titrated

and checked. Many of these sera possess natural agglutinins for *T. lewisi* that may account for their failure to encourage growth. It is inferred from these observations that the action of normal rat serum is specific.

The quantity of rat serum necessary to support the growth of *T. lewisi* appears to vary with the weight of the host. Trypanosomes will grow in 15 g mice with as little as 0.05 ml of normal rat serum/day, thus suggesting that minute volumes supply sufficient material for establishment of the parasite. What proportion is needed for trypanosomes exclusively has not been determined. It is assumed that a certain quantity may be destroyed by the host's body reactions. The minute quantities that appear to be involved here would suggest a nutritional factor possibly on the order of an enzymic protein.

Further support for a nutritional hypothesis comes from observations on the effect of starvation on the quality of rat serum. Rats starved for periods of 12-14 days provide serum that supports little or no growth of the trypanosomes in mice (table). The serum from

*Growth of* Trypanosoma lewisi *in mice supplemented with serum from starved and normal rats*

| Days after inoculation | Average number of trypanosomes per cubic milliliter of blood × 104 | | |
|---|---|---|---|
| | Normal serum | Serum from starved animals | |
| | | 7–9 days starvation | 12–14 days starvation |
| 3 | 0.80 | 2.80 | 0.05 |
| 5 | 10.80 | 4.10 | 0.18 |
| 6 | 6.90 | — | 0.13 |
| 7 | 43.52 | 6.20 | 0.10 |
| 8 | 17.20 | — | 0.50 |
| 9 | 32.70 | 2.70 | — |
| 10 | 29.90 | 2.50 | 0.40 |

rats starved for only 7-9 days supports growth of the parasite intermediate between that observed for normal serum and serum from animals starved 12-14 days. This suggests that the levels of growth-promoting quality of the serum may be changed experimentally by withholding particular dietary components from the rat's diet. This

should permit identification of the substances responsible for this either by selective withholdings from the rat's diet or by specific additions to deficient serum.

We have also attempted to feed normal rat serum to mice in an effort to determine whether the factors responsible for this phenomenon may pass the intestinal mucosa. So far all efforts to secure growth of the trypanosomes in mice given normal rat serum by mouth have failed.

To summarize, then, the rat trypanosome may be induced to grow in the mouse by intraperitoneal administration of normal rat serum to the heterologous host. The infections produced in mice are comparable to those observed in rats, and the trypanosomes may be serially inoculated into mice provided with rat serum. They do not grow in mice without rat serum, nor do they lose their ability to develop normally in rats even after more than 250 subpassages in mice. The working hypothesis developed to account for this phenomenon is that the action of the serum is nutritional providing a specific growth factor to the trypanosomes. This factor is required in minute quantities and may be reduced or eliminated by starvation of the rat.

## BIBLIOGRAPHY

DAVIS, B. D., and DUBOS, R. J. — «Serum albumin as a protective rather than nutritional growth factor in bacteriological media», *Federation Proc.*, **5**: 246, 1946.

LINCICOME, D. R. — «Growth factor in normal rat serum for *Trypanosoma lewisi*», *Jour. of Parasitology*, **41** (6; section 2): 15, 1955.

LINCICOME, D. R. — «Growth of *Trypanosoma lewisi* in the heterologous mouse host», *Amer. J. Trop. Med. Hyg.*, **6** (2): 392, 1957.

POLLACK, M. R. — «The growth of *H. pertussis* on media without blood», *Br. J. Exptl. Pathol.*, **28**: 295-307, 1947.

DESOWITZ, R. S., and WATSON, H. J. C. — «Studies on *Trypanosoma vivax*. I. Susceptibility of white rats to infection», *Ann. Trop. Med. Parasit.*, **45**: 207-219, 1951.

## GENERAL DISCUSSION

Dr. E. Friedheim (U.S.A.) — In contrast to Mel B, Mel W is water soluble and applicable by the subcutaneous and intramuscular route. In the *T. brucei* infections of the mouse it is at least as active as Mel B and less toxic by a factor of 2:

LD50 i.p. = 0.150 g/kg
D cut 100 i.p. = 0.002 g/kg

In human *T. gambiense* sleeping sickness Mel W brings about the disappearance of trypanosomes from blood and lymphglands within the 2 hours following an i.m. or s.c. dose of 2-4 mg/kg. In 2nd stage sleeping sickness a 4 day course of Mel W achieves a rapid fall of the CSF cell-count and the disappearance of the trypanosomes of the CSF, paralleled by prompt clinical recovery.

These findings are illustrated by a moving picture of the day by day improvement of a late second stage sleeping sickness patient, progressing within a week from coma to full consciousness and co-ordinated movements.

*Wednesday, September 10th — 3:00 to 4:30 p.m. Room B*

The Chairman of the meeting was Dr. Fred L. Soper and the Rapporteur Dr. Emmanuel Dias. The Secretary was Dr. Augusto R. Pinto.

The following papers were presented and discussed.

## DOENÇA DE CHAGAS: UM PROBLEMA AMERICANO [1]

EMMANUEL DIAS [2]

Brasil

Os progressos que se vêm realizando no estudo da doença de Chagas, ou esquizotripanose, são de molde a evidenciar cada vez mais sua importância médica, sanitária e social, que dela fazem um dos mais graves problemas de saúde em extensas regiões latino-americanas. Um panorama de conjunto sobre a endemia e seu *contrôle*, apresentado perante os Quartos Congressos Internacionais de Medicina Tropical e de Paludismo (1), pode ser considerado bastante acurado para a época, de maneira que nos actuais Congressos parecerá mais apropriado fazerem-se apreciações sobre alguns aspectos da evolução de nossos conhecimentos na última década e externar conceitos relativos à imperiosa necessidade do desenvolvimento da luta contra a doença no continente.

Nenhum progresso se verificou quanto à nomenclatura do agente etiológico, permanecendo dividida a opinião dos autores, a despeito dos válidos fundamentos em que se baseia o género *Schizotrypanum* Chagas, 1909.

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[1] Trabalho publicado em *O Hospital*, Rio de Janeiro, vol. 55, n.º 1, pp. 57-65.

[2] Chefe da Secção de Inquéritos e Trabalhos de Campo, Divisão de Estudos de Endemias, Instituto Oswaldo Cruz, e do Centro de Estudos e Profilaxia de Moléstia de Chagas, do I. O. C., Bambuí, Minas Gerais; chefe do Posto Experimental de Bambuí, do Departamento Nacional de Endemias Rurais; observador para doença de Chagas da Comissão do Vale do São Francisco.

Grande desenvolvimento sofreu o conhecimento da distribuição geográfica dos hemípteros vectores, da doença e de seus reservatórios, que foram objecto de trabalhos de conjunto (2) que facilitaram uma visão panorâmica da situação do problema, aqui bem delineada pelo co-relator Prof. Dr. Eduardo Del Ponte.

A despeito de se haver verificado um considerável aumento da casuística em diversos países, ainda se pode repetir que «While comparatively few cases have been reported, it seems probable that millions of cases occur from Mexico down to Argentina». (1).

A área de dispersão da infecção humana foi ampliada com o encontro, há muito previsto, de casos agudos no Sul dos Estados Unidos (3), onde também foram observados casos prováveis de esquizotripanose crónica cardíaca (4). Duas amostras humanas de *S. cruzi* isoladas no Texas foram estudadas por Goble (5), assemelhando-se uma delas à amostra brasileira virulenta e outra sendo dotada de pouca infectividade e aparentemente avirulenta. A infecção humana por *S. cruzi* continua restrita ao continente americano, não obstante contar a endemia com, pelo menos, um transmissor cosmopolita.

Um notável impulso foi verificado no estudo da cardiopatia chagásica. Confirmados amplamente os achados iniciais de Carlos Chagas e seus colaboradores, foram eles desenvolvidos em Bambuí (6) e, a partir de 1945 (7), em outras regiões. Existe hoje farta literatura que demonstra sobejamente a significação da esquizotripanose como factor relevante ou principal de cardiopatia em grandes áreas sul-americanas. À descrição do quadro electrocardiográfico peculiar à moléstia e ao emprego corrente da reacção de fixação do complemento com antígenos preparados com culturas de *S. cruzi* deve-se principalmente o grande avanço observado no Brasil e em outros países, depois de 1945, relativamente à forma crónica cardíaca. Merece menção a publicação do trabalho «Chagas Disease» na revista *Circulation* (8), não apenas pelos dados estatísticos e de correlação anátomo-clínica, como por haver contribuído para difundir, em língua inglesa e nos meios especializados, conhecimentos sobre a doença e suas graves consequências, como infecção por excelência cardiotrópica.

A exposição do co-relator Dr. F. Laranja dá expressiva ideia do que representa a cardiopatia chagásica, cuja importância médico-

-social foi muito bem salientada pelo co-relator Dr. Caldeira Brant. Verdade inconteste é que essa importância vem crescendo assustadoramente, à medida que adequadas investigações clínico-epidemiológicas vão sendo realizadas.

Haja vista as pesquisas efectuadas por Rosenbaum & Cerisola (9) no Norte da Argentina, cujos impressionantes resultados merecem ser aqui resumidos. Trabalhando em localidades das províncias de Córdoba e de Santiago del Estero, obtiveram nada menos que 69 por cento de reacções positivas (de fixação do complemento, antígeno de formas culturais de *S. cruzi*) em 515 indivíduos examinados. Calcularam que sòmente nos departamentos em que trabalharam devem existir actualmente 24 791 chagásicos, ou 54 por cento da população total da região. Observaram alterações electrocardiográficas significativas em 23 e 27 por cento das pessoas examinadas em ambas as regiões, o que lhes permitiu calcular que devem aí existir 13 762 casos de miocardite crónica chagásica! Puderam assegurar que a patologia da zona, e particularmente a patologia cardíaca, é amplamente dominada pela doença de Chagas e que a miocardite crónica chagásica é, por muito, a mais frequente das cardiopatias, porquanto de 71 a 83 por cento das cardiopatias diagnosticadas clìnicamente eram de etiologia chagásica. Diante desses e outros resultados, não poderiam os autores deixar de advertir que a simples possibilidade de que achados semelhantes a estes poderão verificar-se em outras muitas regiões argentinas virá a dar à doença de Chagas o carácter de uma verdadeira calamidade nacional. Insistem, pois, com razão, na necessidade de ampliação de tais investigações e de intensificação da luta contra a doença, destacando o descaso com que as autoridades vêm até o momento considerando o problema.

A realização, em Caracas, das Primeiras Jornadas Venezuelanas de Cardiologia, em Novembro de 1957, sob a presidência do Dr. Gilberto Morales Rojas, assinalou grande progresso no estudo da doença de Chagas, ali em plena *étape* cardiológica, com o reconhecimento, que tanto tardou, de que a esquizotripanose constitui o principal factor etiológico de miocardite na Venezuela.

A gravidade dos quadros clínicos da cardiopatia chagásica aguda e da cardiopatia chagásica crónica é corroborada pela severidade das lesões observadas no miocárdio, autorizadamente passadas em revista pelo co-relator Dr. Magarinos Torres. Como contribuição recente e

valiosa ao conhecimento da anatomia patológica da cardite chagásica, cabe mencionar a tese do Prof. C. Mignone (10).

Embora tenha-se acentuado a tendência a se considerarem certas afecções do tubo digestivo, principalmente o megaesófago e o megacólon, como manifestações devidas à doença de Chagas, cumpre observar que parece ainda não haver elementos suficientes para considerar esta uma questão fechada; incidindo com frequência em casos de esquizotripanose em algumas regiões, elas entretanto não se observam em outras, nas quais grassa intensamente a esquizotripanose, inclusive com frequente comprometimento cardíaco. Magarinos Torres (11) considera não sòmente aberta a questão da etiologia do megaesófago, como, por força de argumentos de diversas ordens, admite até como mais plausível outra etiologia, ainda indeterminada, porém não chagásica.

Procedendo ao estudo da distribuição dos triatomíneos em importante região brasileira, o vale do rio São Francisco, Salomão Serebrenick (12) nos dá conta, em interessante trabalho, de suas relações com as condições climáticas.

No terreno da terapêutica, infelizmente, não há progressos sensíveis a serem assinalados, não obstante haver sido incrementado o estudo da acção de drogas em infecções espontâneas e experimentais por *S. cruzi,* sendo digno de lamentar-se o registro de que a esquizotripanose ainda continua sem uma terapêutica etiológica eficaz. Contudo, são dignos de nota os esforços para a pesquisa, em bases científicas, sobre o assunto, empreendidos por Botafogo Gonçalves e colaboradores, apresentados aos Congressos.

Em compensação, no campo da profilaxia pode ser consignado sensível progresso, especialmente no que diz respeito aos métodos de combate aos insectos transmissores. O trabalho do co-relator Dr. A. Limaverde bem mostra que foi amplamente confirmada a notável acção triatomicida do hexaclorociclo-hexano (BHC ou HCH).

Foi desenvolvida uma táctica de aplicação domiciliária (e peridomiciliária) de pó molhável de BHC (com 10 a 12 por cento de isómero gama e em suspensão aquosa a 5 por cento), que possibilita a erradicação dos «barbeiros» com o número mínimo de duas aspersões, feitas a curto intervalo, correspondente a pouco mais que o tempo de incubação dos ovos; a segunda borrifação tem por fim a eliminação das «larvas» eclodidas após a primeira e que devem cons-

tituir o mais importante factor intradomiciliário de reinfestação das habitações submetidas a expurgos a intervalos longos ou a apenas um expurgo. O método foi experimentado no Município de Bambuí pelo Instituto Oswaldo Cruz, com a decisiva colaboração da Comissão do Vale do São Francisco e do Departamento Nacional de Endemias Rurais. Conforme o expõe em seu trabalho a co-relatora Nícia Pinto Dias, os triatomíneos importantes da região, *Triatoma infestans* e *Panstrongylus megistus,* antigamente ali extensa e intensamente dispersos, acham-se hoje em dia pràticamente erradicados de todo o Município.

No sector da profilaxia merecem também ser consignados os meritórios esforços desenvolvidos pelo Ministério da Saúde no sentido de promover a melhoria do tipo mais primitivo de habitação rural, a cafua, *habitat* clássico dos triatomas, medida complementar de valor, mas não decisiva, para a profilaxia da doença de Chagas e, além disto, de grande alcance para a elevação do nível de vida, geralmente ínfimo, das populações rurais das zonas infestadas. As grandes bases em que ainda se esteia a luta contra a doença de Chagas são o combate aos transmissores por meio de insecticidas apropriados, a melhoria da casa rural e a educação sanitária, e é de uma combinação adequada das mesmas que se poderá esperar um resultado ideal.

Quanto à luta contra os hemípteros vectores, embora tenha sido e ainda venha sendo executada com a finalidade do «*contrôle*», e não da «erradicação», não parece haver dúvida a respeito de que este último objectivo, ou conceito, deverá prevalecer sobre aquele. Se já contamos com tácticas eficazes para conseguir a erradicação, não pareceria justificável que persistíssemos no emprego de tácticas de *contrôle,* que exigem custosas aplicações domiciliárias de insecticida em amplas zonas contaminadas, por tempo indeterminado.

Tanto este como outros pontos de vista foram bem aceitos em reuniões e mesas redondas havidas em 1957 em diversas cidades (Buenos Aires, Montevideu, Caracas, Maracay, San Juan de Los Morros, Bogotá, Lima e Santiago do Chile), depois das quais o autor considerou geralmente admitidos os seguintes pontos:

1.º Torna-se cada vez mais evidente a importância médica e social da doença de Chagas e, portanto, a necessidade da aplicação de medidas preventivas e adequadas;

2.º A profilaxia desta endemia deverá ser executada especìficamente, e não secundàriamente, com outras campanhas contra vectores de outras infecções;

3.º Em vista de haverem os triatomíneos permanecido em todas as regiões submetidas a tratamentos domiciliários isolados e larga ou irregularmente intervalados, com BHC ou dieldrin, tal forma de actuar contra esses insectos deverá ser abandonada;

4.º A luta contra os transmissores do *S. cruzi* deve ser sèriamente encarada e deverá ser conduzida com o objectivo de promover a sua *erradicação* de determinadas regiões, que irão sendo ampliadas na medida do possível;

5.º Embora tenha sido unânimemente aprovado o plano de erradicação com base na táctica de duas borrifações integrais, intervaladas de cerca de 30 dias, plano este que será submetido a provas em diversas regiões e contra diversos transmissores, sempre se salientou que ele constitui apenas a primeira palavra em matéria de planos de erradicação de triatomíneos, que sempre deveremos nos esforçar por melhorar, a fim de se conseguir o seu objectivo da maneira mais rápida, segura e económica possível;

6.º Se bem que todo o esforço deva ser desenvolvido no sentido de se promover a melhoria do tipo primitivo de habitação rural, as populações das imensas áreas infestadas por triatomíneos deverão ser libertadas dos transmissores da doença de Chagas, sem esperar pela execução dessas medidas.

Planos-piloto de erradicação já estão sendo executados no Uruguai (13) e no Chile (14) contra *T. infestans* e na Venezuela (15) contra *Rhodnius prolixus*. Foi feito um esforço no sentido de se apresentarem nestes Congressos alguns desses resultados, mas circunstâncias diversas o impediram. A este respeito dirigimos, em Fevereiro de 1958, longa carta ao Dr. Marcolino Candau, ilustre director da Organização Mundial da Saúde, que deixamos de transcrever *in totum* por falta de tempo e espaço. Nela estranhámos que «até hoje não foi organizado um plano de carácter internacional tendente a equacionar sèriamente o problema, particularmente o de sua profilaxia, que é, sem dúvida, o de maior relevância, por se tratar de moléstia grave, ampla e intensamente difundida, para a qual ainda não encontrou a ciência uma solução terapêutica», não obstante sugestões ou

recomendações redigidas em diversos certames científicos internacionais. Continuámos: «A despeito disto, não há, ainda, sinais de uma coordenação efectiva, tendente a focalizar devidamente o problema [...] Aparentemente não será mais possível que os organismos sanitários continuem negligenciando este magno problema de saúde pública, persistindo em lamentável falta. Enquanto que milhões são gastos com problemas que, embora importantes, já foram superados do ponto de vista sanitário, a doença de Chagas continua pràticamente ignorada por eles.» Foram então submetidas ao director da O. M. S. as seguintes sugestões:

«1) Esposando o ponto de vista de que a luta contra os «barbeiros» deva ser conduzida com o objectivo da completa eliminação deles, faça promover uma reunião de especialistas, a fim de que apresentem planos para erradicação dos principais vectores domiciliários da esquizotripanose;

2) Proporcione os recursos indispensáveis à realização de campanhas-piloto contra esses vectores, com base nas conclusões alcançadas nessa reunião;

3) De conformidade com o resultado dessas campanhas-piloto, promover ensaios de erradicação em áreas progressivamente mais amplas de diversos países, sem nunca perder de vista a possibilidade de aperfeiçoamento do método de combate;

4) Não obstante pareça difícil, remota ou quimérica a obtenção do objectivo ideal, que é a eliminação desses hemípteros de toda a América ou apenas das áreas mais cruelmente flageladas pela endemia, esse objectivo não poderá deixar de ser, mais cedo ou mais tarde, encarado, e nada impede que o seja desde agora, o que demonstrará o alto discernimento esperado das maiores autoridades sanitárias do mundo;

5) Assim sendo, desde já poderá cuidar a Organização Mundial da Saúde de constituir serviços nacionais de erradicação de transmissores da doença de Chagas, nos moldes dos criados para outras endemias;

6) Finalmente, que seja aproveitada essa grande oportunidade da comemoração de meio século da descoberta de Carlos Chagas para serem dados passos decisivos pela Organização Mundial da Saúde e pela Repartição Sanitária Panamericana,

a critério de seus ilustres chefes, passos esses que, pela sua transcendência, hão-de converter-se em benefício da mais alta valia para a saúde do homem e o progresso da América.»

A missiva termina com a alusão à necessidade de uma luta que, em sã consciência, não pode ser adiada por mais tempo, referindo-se também ao carácter traiçoeiro da doença, que, intratável mas evitável, tem conseguido permanecer até agora disfarçada como problema secundário aos olhos de muitos daqueles sobre cujos ombros pesa a responsabilidade de proteger a saúde das colectividades.

Poderá ser considerado com o facto mais grave e lamentável da última década o alheamento pràticamente total da Repartição Sanitária Panamericana do problema, a despeito dos múltiplos apelos e recomendações que lhe têm sido endereçados, inclusive como conclusões formais de certames científicos importantes.

Na sua alocução de 7 de Abril de 1953, comemorativa do transcurso do primeiro decénio da Organização Mundial da Saúde, Fred L. Soper pôde assinalar a prodigiosa mudança de estratégia, «que passou do mero *contrôle* das enfermidades à ofensiva activa contra as mesmas, tendo-se a erradicação completa como objectivo na solução de certos problemas sanitários específicos», afirmando, talvez com demasiada ênfase: «À medida que se vão coroando de êxito os programas de erradicação ora em curso, outras enfermidades, é de esperar-se, revelar-se-ão como erradicáveis, e erradicadas serão, em seguida.» Confiemos em que não continue tardando mais o dia em que comecem a concretizar-se, em relação ao grave problema americano da esquizotripanose, estas confortantes palavras do director da Repartição Sanitária Panamericana, certamente um dos maiores pioneiros em matéria de erradicação de vectores de doenças.

## BIBLIOGRAFIA

1. Dias, E., & Laranja, F. S. — «Chagas' disease and its control», IV Internat. Congr. Trop. Med. Mal., Washington, 1948, Abstr.: 91-92, *Proceedings*, 2: 1159-1170, 1948.
2. Dias, E. — «Chagas-Krankheit (Chagas' disease)», *Welt-Seuchen Atlas, Falk Verlag*, Hamburg, 2: 135-140, 1956.

3. Woody, N. C., & Woody, H. B. — «American Trypanosomiasis (Chagas' disease). First indigenous case in the United States», *J. Am. Med. Ass.*, **159**: 176-177, 1955.
4. Dias, E. — «Doença de Chagas nas Américas. I — Estados Unidos», *Rev. Brasil. Mal. D. Trop.*, **3**: 448-472, 1951.
5. Goble, F. C. — *A comparison of strains of* Trypanosoma cruzi *indigenous to the United States with certain strains from South America,* VI Congr. Intern. Med. Trop. Pal., Lisboa, Setembro, 1958.
6. Dias, E., Laranja, F. S., & Nóbrega, G. — «Doença de Chagas», *Mem. Inst. Oswaldo Cruz,* **43**: 495-582, 1945.
7. Laranja, F. S. — «Evolução dos conhecimentos sobre a cardiopatia da doença de Chagas. Revisão crítica da literatura», *Mem. Inst. Oswaldo Cruz,* **47**: 605-669, 1949 (1951).
8. Laranja, F. S., Dias, E., Nóbrega, G., & Miranda, A. — «Chagas' disease. A clinical, epidemiologic and pathologic study», *Circulation,* **14**: 1035-1060, 1956.
9. Rosenbaum, M. B., & Cerisola, J. A. — «Encuesta sobre enfermedad de Chagas en el Norte de Cordoba y Sur de Santiago del Estero», *La Prensa Médica Argentina,* **44**: 2714-2727, 1957.
10. Mignone, C. — *Alguns aspectos da anatomia patológica da cardite chagásica crónica.* Tese para a cátedra de Anatomia Patológica da Fac. Med. da Univ. de São Paulo. 238 pp., 1958.
11. Magarinos Torres, C. B. — Relatório ao II Congr. Latino-Americano de Anatomia Patol. e II Reunião da Sociedade Brasil. de Patologia, São Paulo, 7 a 13/9/58. Inédito.
12. Serebrenick, S. — *Distribuição geográfica dos triatomíneos no vale do São Francisco e sua relação com as condições climáticas,* VI Congr. Int. Med. Trop. Pal., Lisboa, Setembro, 1958.
13. Lopez Fernandez, J. — Comunicação pessoal, 1958.
14. Neghme, A. — Comunicação pessoal. C. F. «Programa experimental de erradicación del *Triatoma infestans*», *Bol. Chileno Parasit.*, **13**: 25, 1958.
15. Torrealba, J. F. — Comunicação pessoal (16-12-57).

# IMPORTÂNCIA MÉDICO-SOCIAL DA DOENÇA DE CHAGAS

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A doença de Chagas é uma infecção de carácter endémico, de evolução crónica, transmitida por hemípteros reduvídeos hematófagos e causada por um hemoflagelado da família *Trypanosomidae*, o *Schizotrypanum cruzi*, Chagas 1909 (9). Suas formas clínicas são: *a*) forma aguda; *b*) formas crónicas, correspondendo a 1) forma cardíaca (cardiopatia crónica) e a 2) forma indeterminada (cardíacos potenciais) (10).

Köberle, professor de Anatomia Patológica, acha que o *S. cruzi* actua no organismo por uma neurotoxina que, destruindo as células nervosas, dá origem aos distúrbios observados nos megas. Outros autores, entretanto, não concluíram ainda por uma relação etiológica entre o *S. cruzi* e os numerosos casos de megacólon e megaesófago observados nas áreas chagásicas.

A moléstia existe desde o Sul dos Estados Unidos (Texas) até à cidade argentina de Nemquem, no paralelo 38, tendo sido encontrada no México e em quase todos os países da América Central e do Sul, com excepção das Honduras e das Guianas Inglesa e Holandesa (19).

Nas áreas endémicas do Brasil, Argentina e Venezuela vivem, aproximada e respectivamente, 15 000 000, 3 500 000 (16) e 2 000 000 (18) de pessoas expostas à doença.

Na Argentina, sobre uma população total de 418 431 habitantes existentes nos departamentos de Córdoba e Santiago del Estero e nas províncias de La Pampa e La Rioja, se calcula que há cerca de 125 100 indivíduos infectados e 23 473 casos de miocardite crónica chagásica (17).

Eis mais alguns dados parciais sobre a incidência de casos humanos em diversos países: Chile, 2239 (6); Bolívia, 218 (5); Equador, 206 (7); Paraguai, 14 (5); Peru, 1 (7).

América Central: 140, distribuídos por Guatemala, El Salvador, Nicarágua, Costa Rica e Panamá (4).

No Brasil, para se avaliar a relevância do problema, citamos: o extinto S. N. M. encontrou triatomíneos em 18 279 localidades, dentre 31 894 inspeccionadas. O *Triatoma infestans* foi achado em 317 municípios, o *P. megistus* em 320, o *T. sordida* em 189, o *T. brasiliensis* em 73, o *T. maculata* em 65. Os índices globais das espécies por estados foram: Rio Grande do Sul, 44,59 por cento; Paraná, 17,46 por cento; São Paulo, 28,14 por cento; Minas Gerais, 25,02 por cento; Goiás, 20,80 por cento; Baía, 19,32 por cento; Pernambuco, 12,29 por cento; Ceará, 4,34 por cento; Paraíba, 3,12 por cento; Rio de Janeiro, 3,28 por cento.

Por espécies temos os seguintes números de exemplares examinados e índices de infecção: *T. infestans,* 75 661 (27,69 por cento); *P. megistus,* 25 173 (15,28 por cento); *T. sordida,* 5907 (9,65 por cento); *T. brasiliensis,* 1396 (8,52 por cento); *T. maculata,* 949 (3,27 por cento).

Ainda pelo S. N. M., realizaram-se inquéritos sorológicos em 7 estados e 23 municípios, examinando-se indiscriminadamente 17 265 indivíduos e encontrando-se 3626 (21 por cento) chagásicos. Em 16 municípios de 4 estados (áreas endémicas) 4330 pessoas não seleccionadas foram submetidas a exames electrocardiográficos, constatando-se entre elas 1026 (23,7 por cento) com alterações miocárdicas. Em 1395 electros de pessoas com R. G. M. positiva 93 continham distúrbios tradutores de cardiopatia chagásica.

Cerca de 3 000 000 de casas se situam em zonas triatomínicas (1)! Isto significa que aproximadamente 15 000 000 de brasileiros estão expostos a esta endemia.

Só E. Dias, em Bambuí e imediações (M. Gerais), tem perto de 3000 casos confirmados. Nessa mesma área, Laranja e colaboradores estudaram 1520 casos: 180 de infecção aguda, 657 com infecção crónica assintomática e 683 com cardiopatia crónica chagásica. Jairo Ramos e colaboradores (1949), em Cássia dos Coqueiros (S. Paulo), em 500 habitantes não seleccionados acharam 45 cardíacos. Em 1949 Laranja, após extensa revisão crítica da literatura sobre cardiopatia

chagásica, afirma que até fins de 1944 o número de casos desta cardiopatia publicados no Brasil fora de Lassance era de 45 e no estrangeiro de 134 e logo depois conclui: mais de 600 casos da referida cardiopatia foram publicados no Brasil nestes últimos 4 anos (11).

Ainda em Bambuí, onde cerca de 50 por cento dos chagásicos crónicos são cardíacos (12) e a mortalidade geral (agudos e crónicos) foi de 81 por cento antes dos 50 anos (14), de 235 casos, 22 (9,4 por cento) morreram durante o período agudo com meningoencefalite, insuficiência cardíaca ou complicações (13). Apesar de o prognóstico parecer favorável na maioria dos casos agudos, «pouco poderá ser dito quanto ao futuro de tais doentes». Não sabemos precisamente quanto tempo depois da fase aguda advirá a cardiopatia crónica. Esta constitui a expressão clínica por excelência da forma crónica, que, por sua vez, tem grande importância por abrigar a imensa maioria dos casos da doença.

Dentre as várias formas crónicas, existem no momento actual evidências seguras ùnicamente em relação à realidade da forma cardíaca; as demais ou não se impuseram à aceitação generalizada ou esperam provas que lhes demonstrem a realidade etiopatogénica (10).

A cardiopatia crónica se assenta em bases: epidemiológicas, clínicas, electrocardiográficas, laboratoriais, anátomo-patológicas e experimentais.

Laranja assevera: «Nosso conceito actual da cardiopatia chagásica crónica é o de um tipo de cardiopatia progressiva, usualmente grave, relacionada à infecção crónica pelo *S. cruzi*, afectando preponderantemente homens nos grupos etários dos 20 aos 50, manifestada clìnicamente pela ocorrência quase constante de distúrbios na formação e condução de estímulo cardíaco e pela insuficiência cardíaca congestiva. Alterações inflamatórias anatòmicamente disseminadas do miocárdio são comummente acompanhadas por lesões circunscritas do endocárdio parietal e por alterações isquémicas do miocárdio, que se desenvolvem vagarosamente.» (13).

É pois principalmente do comprometimento cardíaco que decorre a importância médico-social da doença de Chagas.

Muito difundida em Minas Gerais, esta cardiopatia existe com características semelhantes em S. Paulo, Baía, Pernambuco e Goiás (2).

O que confere especial gravidade à moléstia de Chagas é também a ineficácia da sua terapêutica. De todos os remédios empregados para curá-la nenhum deu resultado.

Ressaltando a enorme significação médico-social desta doença, importa sobremaneira frisar que o problema é ponderável e sério na América Latina e precisa urgentemente de ser encarado com a decisão que sua gravidade exige, pois incide em grande parte de seu território, ocasiona grande número de cardiopatias, principalmente na zona rural e em grupos etários mais produtivos (20 a 50 anos), reduz a duração média da vida, incapacita para o trabalho numerosa parcela da população interiorana e acarreta com frequência mortes súbitas de trabalhadores jovens, constituindo, desse modo, considerável entrave à produção agrícola e industrial e desastrosa consequência para a economia da América Central e do Sul.

A aquisição da cardiopatia chagásica está intimamente vinculada à condição económico-social dos indivíduos que a possuem. Com efeito, a doença de Chagas é contraída sobretudo por indivíduos de inferior nível económico-social do nosso interior, em geral os que habitam o rancho, com seu reconhecido cortejo de deficiências higiénicas e sanitárias (17).

O facto de que alguém adoeça gravemente do coração em decorrência da pobreza material e intelectual do meio em que vive denuncia desde já um problema social cuja magnitude depende da percentagem de casos no total da população. A ubiquação do problema chagásico na América Latina e no conjunto de nossos problemas sanitários e sua ulterior repercussão social e económica dependem pois, em última análise, do número total de infectados que venham a ter cardiopatia chagásica (17).

A miséria e a ignorância constituem, sem dúvida, os factores mais favoráveis à propagação da endemia chagásica. A cafua, rancho, ou mocambo, construção primitiva com paredes de barro batido ou adobe, e geralmente coberta de palha, é o tipo predominante de habitação do nosso sertanejo. De pouca altura, mal iluminada e sem nenhum conforto, proporciona fácil promiscuidade entre seres humanos e animais, inferiorizando o homem que aí vive. Cheia de numerosas frestas e frinchas em suas paredes toscas de barro seco, constitui essa mais primitiva e anti-higiénica moradia o criadouro ideal e permanente de triatomíneos (15).

Já se tem dito e repetido com muita propriedade que combater a cafua é combater a doença de Chagas.

Foi por isso que Mário Pinotti, à frente do D. N. E. Ru., teve o grande mérito de adoptar em alta escala o processo de revestir com cuidado, interna e externamente, as paredes das cafuas com argamassa, de baixo custo e fácil aplicação, feita com areia, barro e estrume de gado.

Esse processo, empregado desde a época colonial em alguns locais do nosso interior, sobretudo em Minas Gerais, e que foi usado também por Cecílio Romaña, na Argentina, garante por longo tempo paredes lisas sem rachaduras e, consequentemente, sem barbeiros. Estamos certos de que a difusão de tal método em áreas endémicas dará os resultados mais auspiciosos, permitindo-nos para o futuro pensar na erradicação da doença de Chagas.

Outra medida que, desde 1950, vem o Governo realizando, em campanhas também chefiadas pelo insigne sanitarista Mário Pinotti, é a profilaxia da doença de Chagas através do combate sistemático ao seu insecto vector, mediante aplicação periódica nos domicílios de B. H. C. em pó molhável, na dose de 500 mg de isómero gama por metro quadrado de superfície.

Registram as nossas estatísticas a realização de mais de 1 500 000 expurgos domiciliares, em milhares de localidades, em centenas de municípios, dos estados de Minas Gerais, S. Paulo, Goiás, Rio Grande do Sul, Paraná e Baía (15).

A terceira arma poderosa no combate à doença e empregada em grande escala e adequadamente pelo D. N. E. Ru. é a educação sanitária.

Congratulando-nos com tantos e tão ilustres luminares da ciência médica universal aqui presentes, pedimos vénia para recomendar nestes magníficos Congressos:

1) Levantamento do nível económico das populações, e nisto está altamente empenhado o ilustre presidente do Brasil, Dr. Juscelino K. de Oliveira;

2) Urgentes e amplas pesquisas para despistamento da doença de Chagas, e sobretudo de sua forma cardíaca, o que deverá ser feito em áreas endémicas, em amostras representativas e indiscriminadas, através de intensos e extensos inquéritos clínico-epidemiológicos;

3) Combate sem tréguas à endemia por todos os meios de que dispomos, incentivando-se a construção de moradias à prova de triatomíneos.

Como justa e saudosa homenagem ao genial Carlos Chagas, que, ao descobrir a doença que lhe traz o nome, construiu «um monumento científico que representa notável conquista da medicina sul-americana», ouçamo-lo com respeito e admiração:

> «Dada a grande extensão da endemia, a difusão dos insectos transmissores em várias regiões do país, a infestação total dos domicílios nas zonas rurais, sem dúvida este problema sanitário oferece dificuldades práticas, todas elas de ordem económica. Ligado, porém, como se acha, ao desenvolvimento do trabalho, à prosperidade da agricultura, ao povoamento do solo, ao aperfeiçoamento da raça de nossos sertões; ligado, ainda, aos deveres de humanidade e de civilização, ao orgulho de um povo, à grandeza moral de uma nacionalidade, certo não nos faltará a energia necessária para encará-lo um dia de modo decisivo, para resolvê-lo de modo proveitoso.» (3).

## RESUMO

1.° Preliminarmente, para que se tenha uma ideia da extensão do problema no Brasil, são referidos alguns dados clínico-epidemiológicos:

*a*) Inquéritos entomológicos mostram que, dos principais transmissores, o *Triatoma infestans* foi achado em 317 municípios e o *Panstrongylus megistus* em 320. Em 75 661 exemplares de *T. infestans* examinados foram encontrados 27,7 por cento de infectados; e em 25 173 exemplares de *P. megistus* a percentagem de infecção foi de 15,3.

*b*) Pesquisas serológicas em vários pontos do país oferecem os seguintes resultados: em 17 265 sangues de indivíduos não seleccionados, de 23 municípios, de 7 estados, foi feita a reacção de Guerreiro-Machado, encontrando-se 3626 positivos (21 por cento);

*c*) Também de indivíduos não seleccionados, de 16 municípios, de 4 estados, foram feitos 4330 electrocardiogramas, encontrando-se 1026 com alterações miocárdicas (23,7 por cento);

*d*) A casuística da doença é bastante elevada no país, bastando citar que só no Município de Bambuí, em Minas Gerais, já estão registrados cerca de 3000 casos.

2.º São apresentados alguns dados sobre a incidência da moléstia em países da América Latina.

3.º Dá-se então especial relevo à importância médico-social da doença:

*a*) Por acometer preferencialmente os indivíduos que vivem na miséria e ignorância, residindo em precaríssimas habitações;

*b*) Como factor incapacitante, atingindo justamente pessoas jovens na fase mais produtiva para o trabalho, eliminando mesmo, precoce e sùbitamente, muitas delas;

c) Como factor negativo, representando um entrave à elevação económica das populações atingidas.

4.º Clama-se para que o problema seja encarado com a decisão que a sua gravidade exige.

5.º Destacam-se as actividades do D. N. E. Ru. no combate à doença, seja pelo expurgo domiciliário com B. H. C. para eliminar os transmissores, seja pela melhoria da habitação rural, de modo a torná-la imprópria à proliferação dos triatomíneos.

6.º Finalmente, recomenda-se: levantamento do nível económico das populações, urgentes e amplas pesquisas para despistamento da doença de Chagas, e sobretudo de sua forma cardíaca, e o combate sem tréguas à endemia por todos os meios de que dispomos, incentivando-se a construção de moradias à prova de triatomíneos.

## BIBLIOGRAFIA


1. Bustamante, F. M. — «Doença de Chagas. Transmissibilidade e profilaxia», *Anais da Faculdade Fluminense de Medicina,* Dezembro de 1956, vol. iv.
2. Brant, T. C., Laranja, F. S., Bustamante, F. M., e Melo, A. L. — «Dados serológicos e electrocardiográficos obtidos em populações não seleccionadas de zonas endémicas de doença de Chagas no estado do Rio Gande do Sul», *Rev. Bras. Malariol. e D. Trop.,* Abril de 1957.
3. Chagas, C. — *Discursos e Conferências.* Conferência realizada na sessão inaugural do VII Congresso Brasileiro de Medicina e Cirurgia Belo Horizonte, 21-4-1912.
4. Dias, E. — «Doença de Chagas nas Américas. III — América Central», *Rev. Bras. de Malariol. e D. Trop.,* **4** (1), 1953.
5. Dias, E. — «Doença de Chagas nas Américas. IV — Bolívia e Paraguai», *Rev. Bras. de Malariol. e D. Trop.,* **5** (1), 1953.
6. Dias, E. — «Doença de Chagas nas Américas. VII — Chile», *Rev. Bras. Malariol. e D. Trop.,* **5** (2), 1953.
7. Dias, E. — «Doença de Chagas nas Américas. V — Equador e Peru», *Rev. Bras. Malariol. e D. Trop.,* **4** (4), 1952.
8. Dias, E. — «Doença de Chagas nas Américas. II — México», *Rev. Bras. Malariol. e D. Trop.,* **3** (4), 1951.
10. Laranja, F. S. — «Aspectos clínicos da moléstia de Chagas», *Rev. Bras. Med.,* **10**: 482, 1953.

11. LARANJA, F. S. — «Evolução dos conhecimentos sobre a cardiopatia da doença de Chagas. Revisão crítica da literatura», *Mem. Inst. Osw. Cruz,* **47**: 605, 1949.
12. LARANJA, F. S., DIAS, E., & NÓBREGA, G. — «Clínica e terapêutica da doença de Chagas», *Mem. Inst. Osw. Cruz,* **46**: 473, 1948.
13. LARANJA, F. S., DIAS, E., NÓBREGA, G., & MIRANDA, A. — «Chagas Disease. A. Clinical, Epidemiological and Pathologic Study», *Circulation,* **14** (6): 1035, 1956.
14. PELLEGRINO, J. — *A Doença de Chagas em Minas Gerais.* Relatório apresentado ao 1.º Congresso da Associação Médica de Minas Gerais. 1951.
15. PINOTTI, M. — 1.º Seminário Brasileiro da Doença de Chagas: *Importância Médico-Social da Doença de Chagas,* Novembro de 1957, João Pessoa, Paraíba.
16. ROMAÑA, C. — «Panorama Epidemiológico de la enfermedad de Chagas en la Argentina, a través de investigaciones sistemáticas», *An. Inst. Med. Reg. Tucuman,* **4**: 27, 1954.
17. ROSENBAUM, M. B. — «Grave problema sanitário — La enfermedad de Chagas», *La Nación,* 18-2-58, B. Ayres.
18. TORREALBA, J. F. — *Cartilla Antichagásica,* Impr. Nac., Caracas 11 pp., 1947.
19. VILELA, E. — «Moléstia de Chagas. Algumas aquisições recentes, em especial relativas à profilaxia», *R. Bras. Mal. e D. Trop.,* **3** (1): 101-121, 1951.

# BIOLOGÍA DE LOS PRINCIPALES VECTORES EN LA ENFERMEDAD DE CHAGAS

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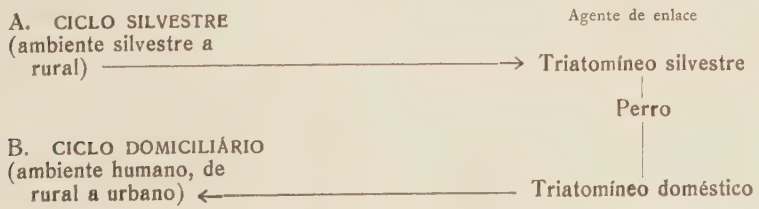
## I

En el ciclo evolutivo del *Trypanosoma cruzi* (o *Schizotrypanum cruzi* según Chagas, Dias y otros autores) intervienen: 1) un trasmisor invertebrado hematófago y 2) un mamífero susceptible. La enfermedad humana es un accidente en este ciclo que, primitivamente, era solo silvestre. No tenemos evidencias perfectas de la existencia actual de este ciclo, pero el frecuente hallazgo de animales agrestes con infección natural, tanto triatomíneos como mamíferos, permite suponer, muy razonablemente, que todavía perdura. Diversas experiencias de laboratorio han mostrado que hemípteros no triatomíneos pueden trasmitir el tripanosoma mencionado, pero solamente los mencionados tienen, por lo menos para el ciclo domiciliario, importancia sanitaria.

El ciclo en el cual interviene el hombre es estrictamente doméstico y ya se ha apartado del silvestre, especialmente en poblaciones más o menos densas, entre rurales y urbanas, pero la relación epidémica puede no haberse perdido totalmente y, en momento oportuno y favorable, el silvestre originar al domiciliario, existiendo entre ambos, como elementos de enlace, dos triatomíneos y el perro; el siguiente diagrama explica esta posible relación:



En amplias regiones americanas, ambos ciclos están perfectamente aislados, y el ciclo silvestre sigue su curso sin interferir con el doméstico pués, en general, los mamíferos y las vinchucas silvestres no abandonan sus biotopos normales a menos que no sean molestados. Quizá *geniculatus* sea un importante trasmisor en este ciclo, dada su gran distribución geográfica, la que señala su adaptibilidad a — también quizá — diversas condiciones ecológicas; Chagas ya la había considerado así en sus investigaciones. De manera que, respecto a una acción sanitaria, puede excluirse totalmente el ciclo A. En otras zonas, donde la enfermedad de Chagas es rara, o no se conoce, y se han encontrado vinchucas con índices de infestación relativamente muy altos, entre las cuales podemos citar los Estados Unidos de Norte América, Méjico, gran parte de Centro América y diversos estados del nordeste brasileño, pareciera que ambos ciclos no fueran tan independientes entre sí, como sucede en la Argentina, Chile, Uruguay, etc. Ello podría atribuirse a que los triatomíneos responsables de los casos conocidos no tienen una eco-etología bien delimitada, entre silvestre y doméstica.

Con estas consideraciones generales por base, fundada en observaciones personales y la abundante bibliografía publicada; presentándose la posibilidad de obtener la infestación experimental con casi todas las especies conocidas y que las infestadas naturalmente son abundantes, aquellas que intervienen en la enfermedad de Chagas son pocas: las adaptadas a la casa del hombre y así los nombres vernáculos que estas reciben, en diferentes países, muestran su relación con el hombre. Puede haber casos especiales, excepciones a esta regla (ver *T. rubrovaria* en Argentina, Uruguay y Rio Grande do Sul); también en zonas donde no hay una nítida separación entre vinchucas caseras y silvestres y donde la dolencia humana no representa, por el momento, un problema sanitario importante.

Les especies importantes para cada país se indican en el cuadro n.º 1, donde agregamos sus índices generales de infestación por *cruzi*. La infestación por *rangeli* está limitada prácticamente al norte de Sud y Centro América y los datos que de ella tenemos no alcanzan a modificar los índices señalados en este cuadro. Gajardo Tobar y Thiermann (1950) han señalado la presencia de *rangeli* en Chile.

En general cada país tiene una o dos especies importantes. En Brasil, dada su gran superficie, se encuentran más especies que en

otros. En algunos estados, como en Minas Gerais, se superponen *infestans* y *megistus* en los bordes de sus respectivas áreas de distribución, actuando ambas como vectores epidémicos. En Méjico se han encontrado varias especies posiblemente vectoras por su hallazgo con infestación natural (81,4 por ciento para *prolixus* y 15,0 por ciento para *dimidiata*), pero los casos humanos conocidos son raros (3 con diagnóstico parasitológico y 5 con R. F. C. positivas, uno de ellos con cardiopatía muy característica); sucede algo semejante en los estados del nordeste brasileño. No podemos dejar de contemplar, para estos casos, la posibilidad de existir cepas locales de *T. cruzi* con menor potencia patógena para el hombre que en Argentina, sur del Brasil, Venezuela, etc.

Todas las especies peligrosas son domésticas, es decir que viven desde el huevo hasta el adulto en viviendas humanas y dependencias adyacentes, como gallineros, cuyeras, árboles circundomésticos, etc. El cuadro n.° 2 y los mapas que acompañan este trabajo muestran la distribución, por países, de las mismas.

Por otra parte, y ello es general para las enfermedades entomotrópicas, el área de distribución de las especies peligrosas es más amplia que la de los casos humanos y seguramente también de la de los mamíferos infectados naturalmente. Es decir que hay áreas potencialmente peligrosas para el hombre y otros mamíferos pero en las cuales no existe (o no se ha comprobado) la infección tripanosómica.

Suprimimos, en este trabajo, la mención de las subespecies; éstas son variaciones geográficas y con indicar la especie creemos cumplido el estudio de este problema. Sin embargo, en algunos casos, las diferentes subespecies pueden tener hábitos distintos y ello puede significar un problema especial: la infestación de *T. protacta* (suponemos que se refiere a la subespecie típica: *protacta*) para Estados Unidos es de 21,9 por ciento y *T. protacta woodi* de 4,8 por ciento (in Dias, 1951*a*).

Creemos conveniente agregar algunas palabras respecto al hospedador vertebrado. Los mamíferos silvestres, fuentes de infección en el ciclo A, quedan, como yá hemos dicho, en su residencia habitual y lo llegan, sino excepcionalmente, hasta la vivienda humana, mientras que los domésticos, que comparten la vida del hombre, son los principales elementos del ciclo B y, en el caso del perro, pueden

frecuentar ambientes silvestres. En Guayaquil (com. pers. de Espinoza) y en el sur del Brasil, la «raposa» o «gamba» *(Didelphis azarai)* vive en los techos de las casas y es un elemento importante en el ciclo domiciliario. Ella ha sido también encontrada infectada en Uruguay y Argentina.

Las especies con infección natural pertenecen a diversos órdenes, entre los que predominan los carnívoros, edentados y roedores; los *Trypanosomidae* de quirópteros no son *cruzi*. Las aves tampoco albergan este tripanosoma, pero los gallineros tienen importante influencia en la densidad de la población de algunos de estos triatomíneos, pues en este biotopo suele encontrarse (por lo menos para *T. infestans* y otras especies) gran cantidad de vinchucas que se traduce, generalmente, por la invasión de la habitación humana.

El dato principal sobre el cual es fundamentado, frecuentemente, la peligrosidad de una especie es su índice de infestación natural, pero es necesario también que este valor se obtenga por el exámen de gran número de ejemplares y que la especie tenga hábitos domésticos definidos o, por lo menos, con antropofilia demostrada. Podría señalarse, como excepción conocida y comprobada, el caso de *rubrovaria*, cuyos detalles se encontrarán más adelante.

Las especies cuya biología presentamos son:

A. — De mayor importancia sanitaria:

1. *Triatoma infestans*
2. *Panstrongylus megistus*
3. *Rhodnius prolixus*
4. *Panstrongylus herreri*
5. *Triatoma dimidiata*
6. *Triatoma rubrovaria*

B. — De menor importancia sanitaria:

7. *Triatoma brasiliensis*
8. *Triatoma maculata*
9. *Triatoma sordida*
10. *Panstrongylus geniculatus*

De cada una de ellas indicaremos, en forma lo más breve posible, su distribución geográfica, desarrollo, hábitos e infestación por *cruzi* o *rangeli*. Hemos procurado obtener las datos más precisos sobre el calendario del desarrollo, considerando que indica la época oportuna para la profilaxis de esta enfermedad. Las ninfas jóvenes son más sensibles a los insecticidas que las mayores o los adultos.

Respecto a las especies norteamericanas no podemos dar estos detalles, por cuanto, siendo la enfermedad de Chagas prácticamente desconocida en Méjico y Estados Unidos, sería pretensioso señalar cual o cuales son las especies que intervienen en el ciclo B, que se desconoce. Los triatomíneos con infestación natural son abundantes y algunos de ellos con índices bastante altos. Los detalles respecto a estos dos países pueden encontrarse en Dias (1951*a* y *b*).

### 1. *TRIATOMA INFESTANS* [1]

*Distribución geográfica* (mapa n.° 1) — Abarca gran parte de la región austral sudamericana, entre los paralelos 12° (Lima) y 35° (Chile)[2] o 44°45′ (Argentina) (Jorg, 1957). Su límite norte forma una ligera convexidad hacia el sur, partiendo del SW peruano, pasando por el norte del Paraguay y luego desciendo verticalmente, desde Minas Gerais hasta Porto Alegre (Rio Grande do Sul). Ha sido citada para el estado de Baía (Brasil). Se la ha encontrado hasta los 4.000 m de altura (Niño, 1929); Mazza la ha hallado en Yavi Chico (Jujuy, Argentina) a 3.800 m y Herrer (1955*a*) a 3.000 m en Ticaco (Perú).

*Desarrollo.* — Ha sido bien estudiado por investigadores argentinos, brasileños, chilenos y uruguayos, por su importancia sanitaria.

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[1] En general, todas las especies aquí consideradas tienen hábitos muy semejantes a *infestans*, de manera que el estudio detallado de ésta nos permitirá ser más breves en el de las restantes. Por otra parte, creemos que es la especie mejor conocida en muchos de sus aspectos biológicos.

[2] Según Gasic (1943); Porter (1938) considera que el límite sur es el paralelo 40° en la provincia de Valdivia.

Las principales observaciones de Hack (1955) hechas en laboratorio, en Resistencia (Chaco, Argentina), están incluídas en el cua-

dro n.° 2; agregamos los datos de Perlowagora Szumlewicz (1953), que hemos colocado entre paréntesis:

CUADRO N.º 2 — *Datos biológicos de* T. infestans [1] *(Hack, 1955; Perlowagora, 1953)*

| Ecdisis entre los días | Resistencia al ayuno | Observaciones |
|---|---|---|
| 1.ª muda . . . . 9 y 17 días (10–17) | | |
| ninfa II . . . . . | 96 días | En laboratorio, con temperaturas ambientales entre 13,2°C y 28,4°C. Julio a enero. Las ninfas fueron alimentadas una sola vez. Experiencias de Perlowagora: 24–28°C. |
| 2.ª muda . . . . 18 y 31 días (18–31) | | |
| ninfa III. . . . . | 17 días | |
| 3.ª muda . . . . 28 y 60 días (31–45) | | |
| ninfa IV. . . . . | 235 días | |
| 4.ª muda . . . . 44 y 81 días (44–66) | | |
| ninfa V . . . . . | 239 días | |
| 5.ª muda . . . 67 y 101 días (67–101) | | |
| adulto macho . . . . . | 146 días | |
| adulto hembra . . . . . | 141 días | |
| Ciclo huevo-adulto . . . . . | 134 días | |
| Ciclo huevo-huevo . . . . . | 187 días | |

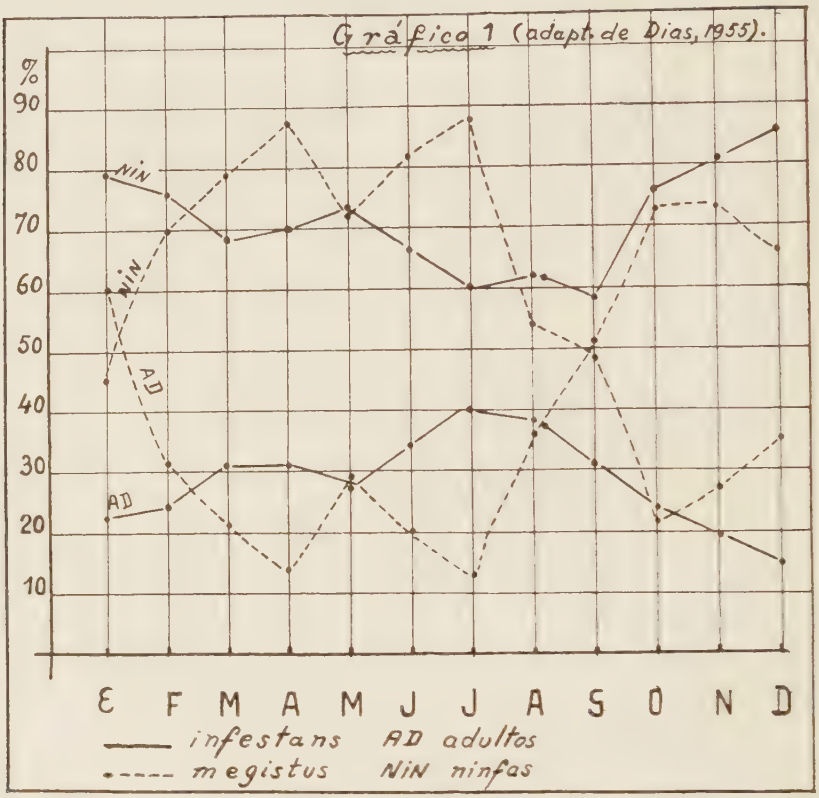
[1] Incubación del huevo { temper. 20° y humedad relativa mayor de 80 % . . . . 27–46 días; temper. 33° y humedad relativa 36 % . . . . . 11–33 días

Gajardo Tobar (1952), en Chile, señala una resistencia al ayuno de 90 a 395 días (larvas); o 151 a 183 días (adultos).

Hack supone que en la parte más templada de la Argentina las vinchucas tienen, «... por lo menos en la gran mayoría, dos generaciones al año, como lo supone Romaña». Dias (1955), en observaciones hechas en Bambuí (Minas Gerais, Brasil), encuentra un predominio de adultos entre abril y agosto; entre septiembre y febrero prevalecen las ninfas ápteras (estados I-IV) sobre las aladas (ninfas V) y adultos. Parece que la producción de ninfas es contínua durante todo el año, posiblemente con más de una generación anual; las observaciones fueron hechas entre los años 1945-47. Este investigador presenta un interesante gráfico (gráfico 1) sobre el porcentaje mensual de adultos y formas jóvenes; de un total de 147.286 vinchucas capturadas, 138.905 (93,9 por ciento) corresponden a *infestans* y el resto (6,0 por ciento) a *megistus*.

Talice y col. (1940), en Uruguay, consideran que la mayor actividad de *infestans* (alimentación y postura) se realiza durante la

estación cálida y que, durante el otoño, ella presenta una última época de actividad, previa al periodo invernal, el cual, por otra parte, es relativo. Estas consideraciones han sido hechas en relación con la curva epidemiológica de la enfermedad de Chagas, referida ésta al comienzo aparente de la misma.




*Hábitos.* — Se encuentra siempre en compañía del hombre, utilizando sus viviendas personales o las de los animales domésticos. Su hallazgo en ambientes silvestres es tan poco frecuente, que los casos conocidos pierden importancia sanitaria ante su estrecha adaptación a la vivienda humana. Se cria, en gran abundancia, en las rendijas, grietas, huecos de paredes y techos de barro (adobe) o paja, detrás de papeles o almanaques o láminas sujetas a las paredes, en cajones o trastos y baúles, etc., tan comunes que se encuentran acumulados en los ranchos. Los huevos son puestos aisladamente,

posible signo de adaptación a este ambiente, en los mismo lugares donde se refugian las ninfas y adultos.

No tienen grandes exigencias alimentarias, atacando tanto a mamíferos como a aves domésticas. Esto último permite encontrarlas, a veces en gran cantidad, en los gallineros y — dadas las costumbres de los pobladores argentinos — no se debe dejar de lado, en un plan de erradicación, los árboles circundomésticos donde acostumbran dormir las gallinas.

Corrêa y Aguiar (1952), en São Paulo (Brasil), y Mayer y Alcaraz (1955), em Resistencia (Chaco, Argentina), han obtenido los siguientes resultados al estudiar las preferencias alimentarias por el método de las precipitinas (cuadro n.º 3):

CUADRO N.º 3 — *Preferencias alimentarias de* T. infestans *en São Paulo y Resistencia*

| | São Paulo | | Resistencia | |
|---|---|---|---|---|
| Totales positivos . . . . . | 631 | — | 437 | — |
| *a*) Hombre, con o sin perro, gato o gallina . . | 361 | 57,2 % | 348 | 79,6 % |
| *b*) Perro o gato, con o sin gallina . . . . . | 182 | 28,8 % | 63 | 14,4 % |
| *c*) Gallina solamente. . . . . | 88 | 13,9 % | 26 | 5,9 % |

La sangre humana no es, por lo tanto, el único alimento buscado y las diferencias observadas entre São Paulo y Resistencia pueden muy bien ser debidas a distintas procedencias de los lotes estudiados. El grupo de vinchucas que se alimentaron únicamente en gallinas tiene importancia lateral en la difusión de la enfermedad, que consiste principalmente en el aumento del tamaño de la población de estos hemípteros e invasión consecuente de la casa humana. Las aves no albergan el tripanosoma patógeno y por ello es general no encontrar vinchucas infestadas en los gallineros o con bajos índices de infestación si se hubieran alimentado antes en mamíferos depositarios. Alimentándose solamente en hombre, las vinchucas también pueden infestarse, pero solamente en un proceso agudo o, si es crónico, con parasitemia, la que, demostrada repetidas veces por el xenodiagnóstico, presenta siempre un porcentaje relativamente bajo, bastante inferior al valor real de la endemia chagásica.

En algunas comunidades del Perú y Bolivia se crian cobayos en viviendas especiales denominadas *cuyeras* o *cocinas conejeras;* ellos

sirven para el consumo humano. En el SW del Perú, Herrer (1955*a*) ha revisado 297 cobayos en distintas localidades, encontrando de 0 a 34,6 por ciento (9 sobre 26) infestados con *cruzi*. Al mismo tiempo encontró un solo gato positivo (1/8 examinados) y ninguno entre 32 perros, 14 conejos y 5 cerdos. El cobayo es uno de los mamíferos domésticos más abundantes en la región mencionada: 83,0 por ciento (776/934); el resto corresponde a perros (9,4 por ciento), gatos (3,3 por ciento), conejos (2,8 por ciento) y cerdos (1,4 por ciento) (Herrer, 1955*b*). Señala también este autor la mayor abundancia de «chirimachas» (nombre vulgar de esta especie) en las cuyeras con respecto a los ranchos y otras dependencias y con mayores índices de infestación (observaciones realizadas en 4 localidades, entre enero y marzo de 1952). Todo ello muestra la estrecha adaptación de *infestans* a este pequeño roedor indígena, más que directamente al hombre; son quizás las condiciones ambientales (obscuridad y tranquilidad) que reinan en ellas muy favorables para su multiplicación. Según los datos publicados (1955*a*; cuadros n.os 2 y 3), se han encontrado (cuadro n.° 4):

CUADRO N.° 4 — T. infestans *encontradas en viviendas humanas y animales en el SW del Perú* (*Herrer, 1955*a)

| | Viviendas revisadas | | | Triatomas infestadas | | |
|---|---|---|---|---|---|---|
| | Revisadas | Con chirimachas | | Promedio de capturas | 1 | 2 |
| Habitaciones humanas . . | 511 | 102 | 19,9 % | 5,9 | 18,8 % | 35,1 % |
| Cuyeras . . . . . | 231 | 77 | 33,3 % | 23,5 | 50,9 % | 73,5 % |
| Gallineros . . . . . | 108 | 31 | 28,7 % | 13,0 | 5,3 % | 6,6 % |
| Corrales diversos . . . . | 97 | 23 | 23,7 % | 13,9 | — | — |

1. En los valles de Caplina, Sama, Socama y Moquega, 1952.
2. En los valles de Vitor y Siguas, 1943.
Es posible que los mayores indices encontrados en 2 sean debidos a la menor cantidad de vinchucas revisadas.

Herrer hace notar que, debido a los hábitos de gallinas y perros de dormir generalmente al aire libre, ellos son menos atacados por las vinchucas que los cobayos. En la Argentina, Chile, Uruguay y Brasil no existe la costumbre indígena precolombina de criar cobayos, pero en estos países, en sus zonas cálidas, también los perros y

gallinas duermen frecuentemente al aire libre. Hace también resaltar la falta de relación de *infestans* con animales silvestres y supone que es de reciente llegada al Perú, no habiéndosela encontrado infestada en la ciudad de Arequipa en 1943, situación distinta a la del año 1952.

En Bolivia, Torrico (1950) halla que *infestans* es muy abundante en las cocinas conejeras, con altos índices de infestación (72,5); entre 38 cobayos estudiados ha encontrado 17 con xenodiagnósticos positivos.

Excepto en estos casos, en las demás áreas de su distribución geográfica no hay rancho o gallinero que carezca de vinchucas, en mayor o menor abundancia. Esta cantidad disminuye a medida que descendemos hacia el sur o ascendemos en las regiones montañosas o nos internamos en ambientes muy húmedos. *T. infestans* tiene predilección por las zonas secas y cálidas y en ellas se las puede encontrar aún en las casas más acomodadas, pero lógicamente en menor cantidad. Parece que las casas de madera del estado de Paraná (Brasil) no son muy buscadas por las vinchucas para refugio o cria; ésta es la opinión de Simões (1943) para explicar la poca frecuencia de la enfermedad en dicho estado.

Interesantes observaciones biológicas ha hecho Dias (1956) en material de cria de distintas especies, entre ellas sobre la que aquí tratamos. Es sobre el tiempo que tardan en alimentarse y su defecación; creemos que ellas tienen relación con la mayor o menor facilidad de infestación del hombre.

CUADRO N.º 5 — *Duración de la picadura y defecación de algunos triatomíneos brasileros (Dias, 1956)*

| Especie | Picadura | | Defecación | |
|---|---|---|---|---|
| | % que picaron sin interrupción | Duración media (minutos) | % que defecaron durante o después de picar | Deyecciones en las 3 primeras horas |
| *T. infestans* . . . | 47,5 | 15,5 | 30,0 | 7,1 |
| *T. sordida* . . . | 79,2 | 20,0 | 12,5 | 4,5 |
| *P. megistus* . . . | 82,1 | 22,7 | 22,7 | 3,4 |
| *R. prolixus* . . . | 20,0 | 14,2 | 50,0 | 13,7 |

Los valores de la tercera columna son importantes y coinciden con la mayor importancia sanitaria de tres especies sobre todas las demás: *infestans, megistus* y *prolixus.*

*Infestación por «cruzi».* — Los valores aquí señalados están referidos a esta especie de tripanosomas. La posibilidad de encontrar *rangeli* en el área cubierta por *infestans* (por ejemplo Chile) no hade modificar esencialmente dichos índices.

Dias (1953*c*) presenta un índice general de 25,4 por ciento (41.975/165.322 examinadas). En diferentes países americanos encontramos:

| | | |
|---|---|---|
| Argentina | 16,6 a 37 % | Dias, 1955*a* |
| Brasil | 21,4 % | |
| Minas Gerais | 18,5 % | Silva Pinto y col., 1952 |
| Paraná | 18,9 % | Sousa Araújo, 1954 |
| Rio Grande do Sul | 40 % | di Primio, 1954*a* |
| São Paulo | 25,2 % | Silva Pinto, 1952 |
| Bolívia | 33,7 % | Torrico, 1950 |
| Chile | 41,0 a 42,8 % | *In* Dias, 1953*b* |
| Perú | 18,8 %[1] / 50,9 %[2] | Herrer, 1955*b* |
| Uruguay | 39 % | Osimani y col., 1950 |

[1] En ranchos.
[2] En cuyeras.

La zona más austral donde se ha comprobado su infestación ha sido en la provincia de Chubut (Argentina), en las localidades de Telsen (1/40) y Gaimán (1/12), sobre el litoral atlántico, en el paralelo 43°15 (Jörg, 1957). En Yaví Chico y la Quiaca (Argentina) ha sido encontrada también con infección natural (Mazza, 1940).

### 2. *PANSTRONGYLUS MEGISTUS*

*Distribución geográfica* (mapa n.° 1). — Su área importante de distribución se encuentra principalmente en el estado de Minas Gerais, extendiéndose desde el estado de Rio Grande do Sul hasta el nordeste del Brasil. Algunos ejemplares han sido hallados en el sur de Mato Grosso (Brasil), Paraguay, Argentina (Corrientes y Misiones) y Guayana Inglesa. En el centro oeste de Minas Gerais se encuentra con *infestans.*

*Desarrollo.* — Neiva (1910) ha estudiado su biología. La incubación del huevo depende de la estación del año; en los meses cálidos es de 25 a 30 días; en los fríos entre 30 y 40 días. En general todos los huevos son fértiles. La ninfa I se alimenta recién a los 5-8 días de edad y las mudas son influenciables por la alimentación saguínea.

Estas mudas se producen: la 1.ª a los 45 días; la 2.ª a los 2-3 meses; la 3.ª a los 4-6 meses; la 4.ª a los 190 días, y la 5.ª a los 232 días. Las ninfas I-IV no mudan después de un ayuno de mayor de 70 días. La ninfa V es muy activa, alimentándose cada 15 días y picando durante 15-20 minutos. Dias (1956) ha encontrado un valor medio de 22,7 minutos (ver cuadro n.º 5) y el 22,7 por ciento defecó durante este tiempo ó inmediatamente después.

Los adultos hacen posturas parceladas de 1-45 huevos, con un valor medio de 8; las primeras son más abundantes (15-25 huevos) que las últimas (2-4) y pueden poner, por lo menos, en 148 días 218 huevos en 38 posturas (Neiva, *op. cit.*). Generalmente las posturas se efectuan a los 5 días después de la alimentación. Neiva supone que la edad mínima para poner huevos es de 53 días y ha observado una de ellas, capturada durante el desove, vivir 150 días. Una hembra completó su ciclo huevo-adulto (el menor lapso observado) en 260 días y el ciclo huevo-huevo sería, según este mismo autor, 314 días (resultados de sumar 53 días pre-posturales a 261 días observados en una hembra).

Dias (1955), en un estudio muy detallado hecho en Bambuí (Minas Gerais, Brasil), desde 1944 hasta 1947, muestra el predominio de los adultos entre octubre y enero; entre febrero y mayo prevalecen las ninfas I-IV y en junio-septiembre las ninfas V; habría así una sola generación anual. En este trabajo presenta un interesante gráfico (p. 458) mostrando los porcentajes mensuales de formas jóvenes y de adultos, en las capturas de los años 1944-1950. De un total de 138.905 capturados, 8.921 correspondieron a *megistus* (6,0 por ciento), valor que muestra su menor abundancia y quizá menor importancia sanitaria que *infestans*, con un porciento de 93,9. Di Primio (1955) ha dado a conocer una secuencia cronológica de capturas (1951-55) hechas en Rio Grande do Sul, donde se observa un predominio de estas vinchucas en los meses cálidos (noviembre a febrero), disminuyendo, hasta desaparecer, en los meses frios. En este estado brasileño suele haber nevadas durante el invierno.

En ciertas condiciones de humedad las hembras pueden resistir prolongado ayuno; Neiva cita un lapso de 57 días en una hembra recibida en Rio de Janeiro proveniente da la Guayana Inglesa. Los machos son menos activos y menos resistentes a la intemperie y ayuno que las hembras. Aún en condiciones favorables se alimentan y viven menos que las hembras. Las ninfas y los adultos se alimentan con mayor avidez a temperaturas altas; a 14° su apetito disminuye mucho y muestran menor actividad.

*Hábitos.* — Es un insecto de hábitos domésticos, que Neiva, en 1910, suponía ser de reciente adaptación, «por cuanto, colocadas en jaulas con hojas verdes, si desovaban, lo hacían sobre las hojas, aglutinados, como es general en los representantes de esta familia» (trad.). En los ranchos los ponen aislados. Viven en las grietas de las paredes y techos de los ranchos y di Primio (1955) las ha encontrado en árboles circundomésticos; no es una especie suficientemente adaptada a la habitación humana (por lo menos para Rio Grande do Sul).

El adulto puede volar con facilidad en busca de alimento y Neiva (1910) lo ha observado cruzando las calles de las poblaciones; los ranchos nuevos permanecen largo tiempo sin ser invadidos por ellas y si una casa infestada es abandonada por sus moradores, *megistus* desaparece a los 30 días, distribuyéndose por las casas vecinas. En Mato Grosso observó su transporte por las albardas que llevan los jinetes, suponiendo que éste sea el principal medio de difusión de ella.

*Infestación por «cruzi».* — Es muy variable y depende del estado brasileño considerado. Dias (1953*c*) dá un valor general de 27,4 por ciento (8.370/30.542); en Minas Gerais, donde es un importante trasmisor, este valor es de 16,41 por ciento (Silva Pinto y col., 1952). En el área paulista es de 1,4 por ciento (Silva Pinto y Corrêa, 1954), mostrando su menor importancia sanitaria. En Ceará, Machado y Silva Pinto (1952) dan un índice de 8,47 por ciento, pero de 1.247 triatomíneos capturados, solo 83 (6,5 por ciento) eran *megistus,* capturados en 6 entre un total de 115 localidades examinadas; infestadas solamente en 3 de ellas y, en algunas, acompañadas por *T. brasiliensis.* En este estado la enfermedad tiene poca difusión, demostrando ello, una vez más, que no basta el dato de la infestación natural para demostrar la importancia sanitaria de una especie.

### 3. *RHODNIUS PROLIXUS*

*Distribución geográfica* (mapas n.os 1 y 3). — Se encuentra desde el norte del Ecuador y las Guayanas hasta Méjico. Ha sido encontrada también en Bolivia (com. pers. Lent, IX-958). En Centro América ha sido señalada para Guatemala, El Salvador y Panamá (es posible que en este último país sea la especie *pallescens;* cf. Dias, 1952a).

*Desarrollo.* — Uribe (*in* Blanco, 1943) ha hecho algunas observaciones en Guatemala: el período de incubación ovular es, a 30°C, de 8 a 10 días. La hembra se alimenta a los 15 días de eclosionar y puede poner huevos 20 días después. Según Buxton (*in* Blanco, 1943), hembras alimentadas al 3.° y 4.° días y mantenidas a 24°C ponen su primer huevo en el 13.° día. La temperatura óptima es de 30°, poniendo 115 huevos en 60 días, alimentadas 6 veces. La longevidad de éstas, a 30° y comiendo cada 8 días, es de 8 meses, poniendo un máximo de 140 huevos mensuales; no sobreviven muchos días después de la última postura. El acoplamiento puede ser repetido, aún cuando basta una sola cópula para que la hembra quede fecundada para siempre; ésta es fácil de observar, aún en espacio reducido y durante el día. El huevo es puesto aglutinado con los otros y adherido al soporte, recuerdo atávico de los *Reduviidae*. Hay dos generaciones anuales (Dias, 1953c).

*Hábitos.* — Es doméstica y circundoméstica, pero también ha sido encontrada en madrigueras de *Agouti paca* (L.) (=*Cuniculus paca*) y en cuevas de dasipódidos. Pifano (1940), de Venezuela, la cita como encontrada en cuevas de *Coendu prehensilis* infectados.

En Guatemala (Peñalver y col., 1953; Blanco, 1943) se encuentra en las pajas que techan los ranchos, excepcionalmente en las paredes de barro o trastos viejos que nunca faltan en dichas viviendas. En Venezuela, Iriarte (1941) cita un hallazgo en tales petates y no el techo, que era de palma de coco *(C. nucifera);* Pifano (1940) indica como nicho las grietas de las paredes y la trama de palma de los techos. Armas (1944), de Venezuela, señala que *prolixus* tiene preferencia por ocultarse en gramíneas *(Panicum maximus* y *Orundo donax)* y en trojas de maíz.

*Infestación por tripanosomas.* — Debido a la presencia de *T. rangeli,* conviene hacer una distinción entre los tripanosomas que pueden infestar al «pito» (nombre vulgar de *prolixus* en Venezuela).

CUADRO N.º 6 — *Infestación de* R. prolixus *por* cruzi, rangeli *o ambas*

| País | Totales | | | Con *cruzi* | | Con *rangeli* | | Ambas | | Autor e año |
|---|---|---|---|---|---|---|---|---|---|---|
| | Exam. | Pos. | % | Pos. | % | Pos. | % | Pos. | % | |
| Guatemala. . | 70 prep. posit. | | | 46 | 65,71 | 15 | 21,42 | 9 | 12,83 | Peñalver y col., 1952 |
| Venezuela . . | 1.637 | 846 | 51.7 | 454 | 27,7 | 117 | 7,1 | 276 | 16,8 | *In* Dias, 1952*b*. |
| Colombia . . | 247 | 132 | 53 4 | 29 | 11,7 | 49 | 19,8 | 54 | 21,9 | *In* Dias, 1952*b*. |

La infestación tripanosómica de esta especie puede ser muy grande. Pifano (1941), en Venezuela, encontró índices de 76,2 por ciento (1.322/1.735); diversos investigadores encontraron 56,2 por ciento (6.547/11.651) (*in* Dias, 1952*b*).

Esta especie, por su adaptación domiciliaria intensa y sus altos índices de infestación, es la vectora epidémicamente importante para Venezuela, Colombia y países cercanos. En Centro América la enfermedad de Chagas tiene poca difusión.

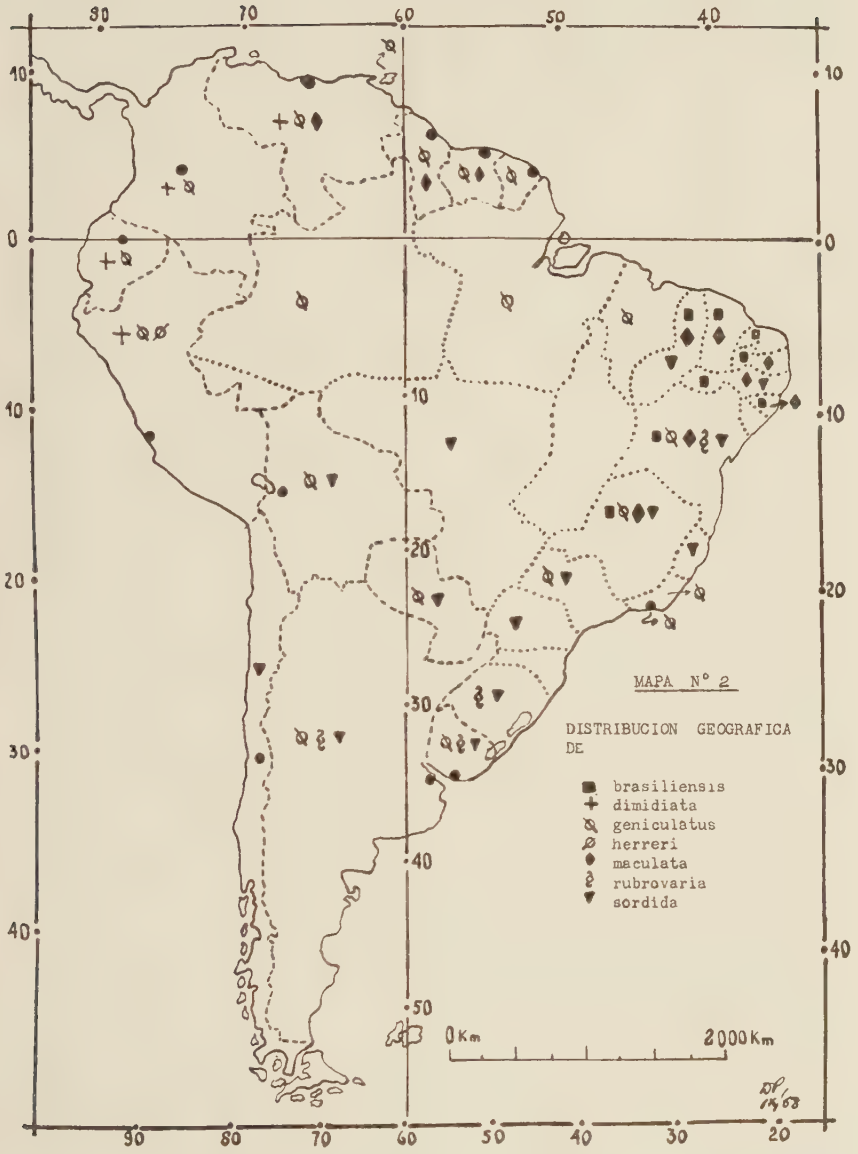
También como en el caso de *T. infestans* y *P. megistus,* el área de dispersión de *R. prolixus* es mayor que el de los casos humanos conocidos (también en Venezuela y Colombia); creemos que estos últimos serán hallados posteriormente en regiones desconocidas a este respecto.

### 4. *PANSTRONGYLUS HERRERI* (= ? *LIGNARIUS*)

*Distribución geográfica* (mapa n.º 2) — Hasta ahora, se conoce solamente del norte del Perú. Se encuentra hasta los 1.450 m sobre el nivel del mar.

*Desarrollo.* — No tenemos datos sobre su desarrollo.

*Hábitos.* — Vive dentro de las casas, con hábitos semejantes a *infestans,* siendo una especie intensamente doméstica. No ha sido encontrada en ambientes silvestres y las búsquedas en los gallineros




han sido casi siempre negativas. No tiene grandes preferencias alimentarias, aunque «prefiere alimentarse en hombre».

*Infestación por «cruzi».* — Resumiendo los valores dados por Herrer en 1955*d* y 1956, para distintas localidades, encontramos 35,2 por ciento (130/369).

Estos datos han sido tomados de Herrer (1955 y 1956).

### 5. *TRIATOMA DIMIDIATA*

*Distribución geográfica* (mapas n.$^{os}$ 2 y 3). — Se encuentra desde el Ecuador y los estados del nordeste brasileño hasta Méjico. Ha sido encontrada también en Minas Gerais (Brasil) y en Perú. En Costa Rica se halla en el litoral pacífico y no en el atlántico, que es muy húmedo.

*Desarrollo.* — Algunos datos han sido publicados por Zeledón (1952) para Costa Rica, Blanco (1943), Peñalver y col. (1953) para Guatemala y Campos (1931) para Ecuador.

El período de incubación parece ser, a temperatura óptima de 30°C, de 12 a 14 días, no desarrollándose el embrión por debajo de 13°. Zeledón dá 21 días, con un óptimo de 28°; entre 21° y 25° este lapso se extiende a 39 días. La postura se inicia al mes de eclosionar la hembra, siendo parceladas, de más o menos 60 huevos, cantidad que aumenta a 25° y con repetidas alimentaciones. Según Usinger (1944; *in* Zeledón), ponen 214 huevos en total; Blanco considera que durante toda su vida este número puede llegar hasta 240 huevos.

La 1.ª muda se hace a los 30 días; la 2.ª a los 2-3 meses; la 3.ª a los 5-6 meses; la 4.ª a los 7-7,5 meses, y la 5.ª a los 9-10 meses. El adulto puede vivir varios meses (Zeledón) o un año (Blanco); son más abundantes en los meses secos, especialmente de enero a marzo (Zeledón). Campos obtuvo un adulto a los 9 meses y 10 días. Ella vive y se reproduce todo el año en Guayaquil, con mayor incidencia en abril a junio (Espinoza, 1955).

*Hábitos.* — Las observaciones publicadas dan la impresión de ser una especie no muy adaptada a la vivienda humana, especialmente comparada con *infestans*. Campos, en 1931, señala que es una especie de adaptación reciente y que 20 años antes (es decir en 1911) no se encontraba con la abundancia que presentaba en 1931. Zeledón considera también que es de reciente adaptación y Pifano (1941), para Venezuela (*in* Zeledón), señala que es una especie principal-

mente silvestre. Campos la ha hallado solamente en los barrios apartados de Cuayaquil, faltos de higiene, especialmente en habitaciones obscuras, húmedas y mal entabladas. De hábitos nocturnos (como casi todos los triatomíneos), vuela atraída por la luz artificial (quizás

comparable ésto con lo observado en *rubrovaria* y *geniculatus*). Este investigador la ha encontrado frecuentemente entre los insectos que acuden a los focos eléctricos, en las primeras noches invernales; *P. megistus,* hallada por de Carlo, en Santo Tomé (Corrientes, Argentina), fué capturada en condiciones semejantes. También vuela dentro de las habitaciones, golpeándose contra las paredes.

Sus hábitos en Costa Rica y Guatemala son semejantes. Las viviendas preferidas son los ranchos (idénticos en su construcción a los argentinos), casas de madera y en biotopos circundomésticos; a diferencia de *infestans,* la cantidad de ejemplares en las casas es

pequeña. En Costa Rica prospera mejor en las regiones cálidas secas (menor período de incubación ovular), desde los 150 m sobre el nivel del mar, en las planicies cálidas de Guanacaste, y se encuentra hasta los 1.360 m en las regiones frescas de la Cordillera Central. En Guatemala se ha encontrado hasta los 1.219 m de altura. En ambos países se refugia en las grietas de las paredes de barro de los ranchos, intersticios de lechos de madera, detrás de papeles, almanaques, etc., colgados en las paredes. Es raro encontrarla entre los techos de paja, pero nó en todas las localidades revisadas por Blanco.

En la actualidad, *dimidiata* es abundante en los barrios suburbanos de Guayaquil (Ecuador), donde se alimenta en *Didelphis azarai,* marsupial que presenta un índice de infestación de 44 por ciento,

Este mamífero vive en las casas con hábitos semi-domiciliarios (Espinoza, 1955). Ello nos muestra una adaptación de esta vinchuca a condiciones tales que la han convertido en un vector peligroso, confirmando la predicción de Campos en 1931.

*Infestación por tripanosomas.* — Los valores conocidos oscilan entre 11,6 y 22 por ciento, pero su posible reciente adaptación a la vida humana hace que ellos no tengan el valor presentado por *infestans, megistus* o *prolixus,* como propagadores de la enfermedad de Chagas. Según Dias (1952*b*), esta especie, después de *prolixus,* es la de mayor importancia sanitaria en Venezuela, con índices locales que varian entre 9,3 y 13,0 por ciento. Excepto en algunas observaciones aisladas, la mayoría de los datos se refieren a su infestación por *cruzi,* sin tener en cuenta la presencia de *rangeli.* Los índices generales para los diferentes países son:

| | | |
|---|---|---|
| Méjico . . . . . | 15,0 % | *in* Dias, 1951*b* |
| Costa Rica . . . . . | 31,6 % | Zeledón, 1952 |
| Guatemala. . . . . | 25,9 % | Peñalver y col., 1953 |
| Colombia . . . . . | 22 % | Hernández, 1947, *in* Dias, 1952*b* |
| Ecuador. . . . . | 26,0 % | Espinoza, 1955 |

En Guatemala, Peñalver y col. encontraron entre 40 preparaciones positivas de deyecciones de *dimidiata:* 120 con *cruzi* (85,71 por ciento), 5 con *rangeli* (3,57 por ciento) y con ambos tripanosomas 15 (10,71 por ciento). En 20 chagásicos (entre 318 personas examinadas: 6,3 por ciento) encontraron: 17 con *cruzi* (85 por ciento), 1 con *rangeli* (5 por ciento) y 2 (10 por ciento) con ambas especies.

Excepto en Venezuela, donde el principal trasmisor es *prolixus*, la enfermedad de Chagas en los demás países citados tiene importancia muy relativa.

### 6. *TRIATOMA RUBROVARIA*

*Distribución geográfica* (mapa n.º 2). — Su área de distribución es bastante llamativa. Su centro endémico está, al parecer, en la zona sudamericana que se extiende desde Córdoba y Entre Ríos (Argentina) hasta Uruguay y el estado de Rio Grande do Sul (Brasil). Neghme (com. person., IX-1958) considera dudoso su hallazgo en Coquimbo, Chile; Lent (com. person. IX-1958) supone que la *rubrovaria* de Java es *migrans*.

*Desarrollo.* — No tenemos datos sobre su desarrollo.

*Hábitos.* — Es una especie agreste, que vive preferentemente en refugios naturales formados por rocas o piedras y donde encuentra su alimento natural en los vertebrados que comparten esta vivienda. Las observaciones de Talice y col. (1940) para el Uruguay, de di Primio (1953) para el estado de Rio Grande do Sul (Brasil), de Toranzos (1944) e Iñiguez Montenegro (1946) para la Argentina coinciden de tal manera que presupone una eco-etiología semejante en todas estas regiones. Estos autores señalan la posibilidad de contraer la infección humana al reposar sobre las piedras y la costumbre de esta vinchuca de «pintas coloradas», como las llaman en el Uruguay, de invadir, durante la noche, las viviendas humanas próximas, en épocas calurosas y antes de las lluvias. Talice y Osimani (1937) comprobaron la infestación natural de *Didelphis azarai* («comadreja overa») y su trasmisión al perro por esta vinchuca. Sería ello un ejemplo demostrativo de la existencia de agentes de enlace que hemos señalado en la primera parte de este trabajo. Iñiguez Montenegro señala un campamento de obreros instalado en el norte de la provincia de Córdoba (Argentina) que ahuyentó la fauna normal de vertebrados de la región y que fué invadido, muy pronto, por esta especie.

La construcción de casas, corrales, muros, etc., con piedras de la zona aumentan las probabilidades del contacto *rubrovaria*-hombre (Talice y col., 1940), con sus consecuencias lógicas. Son menos buscados los ranchos de paja y barro (como sucede con *infestans*). Por

otra parte, puede invadir fácilmente las carpas que sirven de alojamiento a obreros de capamentos más o menos permanentes, como en el caso mencionado por Iñiguez Montenegro, en el cual provocó una epidemia chagásica. Pinto (1942) la ha encontrado en cuevas de *Dasypus hybridus* en Uruguayana (Rio Grande do Sul, Brasil) y ha mostrado su alimentación en *Cavia aperea* («cuis») y *Conepatus chinga suffocans* («zorrino»), el primero circundoméstico y el segundo francamente silvestre.

*Infestación por «cruzi».* — Los datos conocidos son escasos; no habiendo sido hechos sobre cantidad suficiente de ejemplares, los índices publicados pueden ser más elevados que los reales. Para Uruguay encontramos 10 por ciento (Talice y col., 1940) o 34,0 por ciento (Talice y col., 1952: 61/179, en los departamentos de Rivera y Artigas). Di Primio (1954*a*), para Rio Grande do Sul, encuentra 22,2 por ciento; Toranzos (1944), en la Argentina, encuentra 4,95 por ciento.

De las especies restantes, mencionadas en la primera parte (grupo B), hemos encontrado pocos datos biológicos y ecológicos, debido quizás a su menor importancia sanitaria.

### 7. *TRIATOMA BRASILIENSIS*

*Distribución geográfica* (mapa n.º 2). — Hasta la fecha se conoce principalmente en los estados del nordeste brasileño, llegando hasta Baía y Minas Gerais.

*Desarrollo.* — Pocos datos hemos conseguido, correspondiendo principalmente a Pinto (1924 y 1930). Las posturas son parceladas, poniendo huevos aislados. Las ninfas I se alimentan a las 48 horas de nacer y la 1.ª muda se produce, más o menos, a los 45 días. En el nordeste del Brasil comienzan a aparecer en septiembre; en enero sólo por excepción se encuentran ninfas I, encontrándose ninfas mayores o adultos. Para mediados del año las condiciones varían: predominan las larvas y disminuyen los adultos. El ciclo huevo-adulto, en laboratorio, dura aproximadamente 200 días.

*Hábitos.* — Es ya una especie doméstica, aunque se encuentra en corrales de ovejas y cuevas de *Kerodon rupestris*. Abunda principalmente en las regiones secas del Brasil (Dias, 1953*c*). En Ceará,

Machado y Silva Pinto (1952) encontraron entre 1.247 vinchucas, 856 *brasiliensis* (68,80 por ciento); en 1942, Albuquerque, Britos y Morais (según Machado y Silva Pinto, *op. cit.*) solamente el 3,5 por ciento de esta especie. Dicha diferencia puede deberse a distintas localidades examinadas.

*Infestación por «cruzi».* — En Ceará: 53/695: 7,77 por ciento (Machado y Silva Pinto, 1952).

### 8. *TRIATOMA MACULATA*

*Distribución geográfica.* — Venezuela, Guayanas Inglesa y Holandesa y Brasil (principalmente en las regiones secas: estados del nordeste y en Minas Gerais).

*Desarrollo.* — No tenemos datos.

*Hábitos.* — Vive en ranchos de barro, chiqueros y cuevas de «tatús» (dasipódidos). Ataca al hombre y a los animales, especialmente a gallinas y pollos (Dias y Torrealba, 1948). Entre 1.247 vinchucas capturadas en Ceará (Machado y Silva Pinto, 1952), 326 (26,14 por ciento) eran *maculata;* Albuquerque, Britos y Morais (1942; *in* Machado y Silva Pinto, *op. cit.*) hallaron solamente el 4,10 por ciento. Puede atribuirse esta diferencia, como en el caso anterior, a diferentes localidades examinadas. De 105 localidades venezolanas, donde se ha señalado su presencia, solamente 6 han dado ejemplares infestados (*in* Dias, 1952*b*).

*Infestación por tripanosomas (posiblemente «cruzi»):*

| | | | |
|---|---|---|---|
| Brasil . . . . . | 10/522 | 1,9 % | Dias, 1933*c* |
| Paraíba . . . . . | | 1,0 % | Pereira da Silva y col., 1956 |
| | | 3,27 % | Lucena y col., 1953 |
| Ceará . . . . . | | 1,14 % | Machado y Silva Pinto, 1952 |
| Venezuela . . . . . | | 12,2 % | *In* Dias, 1952*b* |

### 9. *TRIATOMA SORDIDA*

*Distribución geográfica* (mapa n.° 2). — Desde Chile, Argentina, Bolivia y Uruguay se extiende por Paraguay, Mato Grosso y Minas Gerais hasta Baía (Brasil).

*Desarrollo.* — Los únicos datos que tenemos son los referentes a la postura, que se inicia a los 30 días de la cópula y que las hembras no fecundadas pueden desovar huevos no fértiles (según Neiva, *in* Pinto, 1930).

*Hábitos.* — Estos datos han sido tomados principalmente de Abalos y Wygodzinsky (1951). Es una especie doméstica en su área de distribución principal y en el NW argentino, pero menos que *infestans* o *megistus*. En la llanura seca de Salta y Santiago del Estero (Argentina) es casi siempre silvestre, encontrándose bajo la corteza de árboles o en nidos de pájaros. En estos casos, se encuentran ejemplares microsomáticos y generalmente más oscuros que los domiciliarios. De la Barrera (1939) la ha encontrado, en Mendoza (Argentina), en cuevas de *Microcavia* y *Galea*, junto con *T. platensis*. Mazza (1936) la encontró en un palomar conejera en J. V. Gonzalez (Salta, Argentina); según Neiva y Penna (1916), *sordida* sería una especie que busca la orilla de los ríos y asi merecería llamarse *fluviatilis*. Ello está en contradicción con las observaciones de Abalos y Wygodzinsky mencionadas, a menos de que se trate de especies distintas.

*Infestación por «cruzi».* — Para Minas Gerais, Silva Pinto y col. (1952) dan un índice de 1.76 por ciento (17/964). Analizando los datos de estos autores (cuadro p. 121), y en mapa respectivo, se encuentra que solamente en 4 municipios, entre 44, encontraron *sordida* infestadas con distintos índices (Itinga, 3/357: 0,84 por ciento; Comercinho, 2/65: 3,07 por ciento; Virgen da Lapa, 6/79: 7,59 por ciento, y Bocaiuva, 3/12: 25 por ciento). *T. sordida* tiene, en Minas Gerais, mayor distribución que *infestans* o *megistus*, pero estas últimas fueron encontradas: *infestans* en 52 municipios entre 82 y *megistus* en 51 entre 114 examinados.

### 10. *TRIATOMA GENICULATUS*

*Distribución geográfica* (mapas n.os 2 y 3). — Desde Argentina y Uruguay hasta Nicaragua, abarcando el este y centro del Brasil, Bolivia, Perú, las 3 Guayanas, Colombia y Trinidad.

*Desarrollo.* — No tenemos datos sobre su desarrollo.

*Hábitos.* — Ha sido encontrada en cuevas de armadillos (dasipódidos), siendo accidental su hallazgo en viviendas humanas. La hemos capturado en Palo Santo (Formosa, Argentina) picando a un caballo, a media noche, en una picada de bosque húmedo a orillas del río Bermejo y sobre nuestra mano, al anochecer, posiblemente atraída por la luz eléctrica.

*Infestación por «cruzi» (o «rangeli)»:*

| | | |
|---|---|---|
| Panamá . . . . . | 10/18 — 55,5 % | Dias, 1953*c* |
| Venezuela . . . . . | 9/51 — 17,6 % | *In* Dias, 1952 |

No nos ha sido posible consultar el trabajo de Bonilla (1938), ignorando si en el mismo hay datos importantes, para nuestro propósito, de esta especie.

## BIBLIOGRAFÍA


Abalos, J. W., y Wygodzinsky, P. — *Los Triatominaes argentinos (Reduviidae Hemíptera)*. Inst. Med. Reg. (Univ. Nac. Tucumán; public. n.° 601). Mon. n.° 2; 178 pp., il., 1951.

Albuquerque y Machado, A. C. — «Contribuição ao conhecimento da nosologia de Alagoas», *Rev. Hig. Saúd. Públ.*, v: 21-39 (citado por Pessoa, 1949), 1947.

Armas, J. — *El problema de la insalubridad rural en el estado de Guárico*. Ed. El Ateneo, Buenos Aires, 189 pp., il., 1944.

Blanco, S. E. — *Contribución al estudio de los reduvídeos hematófagos de Guatemala*. Tesis. Fac. Cienc. Méd., Univ. Nac., Guatemala, 1943.

Bonilla, N. A. — «Reduviidae de Colombia. Un nuevo Triatoma, el *Panstrongylus geniculatus* (Latreille, 1811) (Pinto, 1931)», *Repert. Méd. Cir., Bogotá*, III: 738-746 (no consultado), 1938.

Campos, R. F. — «La chinche sanguinófila *Triatoma dimidiata* Latr. y su amplia dispersión urbana. Peligros que entraña su propagación. Medios para combatirla», *Rev. Col. Nac. V. Rocafuerte*, año XIII (44): 107-111, Guayaquil, 1931.

Chagas Fho., C. — «A moléstia de Chagas no Brasil», *At. XI Conf. San. Panam.*, R. de Janeiro, pp. 240-246, 1942.

Corrêa, R. R., y Aguiar, A. A. — «O teste de precipitina na identificação da fonte alimentar do *Triatoma infestans* (Hemiptera, Reduviidae)», *Arq. Hig. Saúd. Públ.*, XVII (5): 3-8. S. Paulo, 1952.

De La Barrera, J. M. — «Contribución al conocimiento de la peste selvática en la Argentina. Caracteres del brote de Mendoza en 1937», *Rev. Inst. Bact.*, VIII (5): 441, 1939.

De La Barrera, J. M. — «Peste Rural», *Rev. Inst. Bact.*, VII (3): 439-506, B. Aires, 1939.

Dias, E. — «Doença de Chagas nas Américas», *Rev. Bras. Malar. e Doenç. Trop.*, 1951-1955.

1951*a*. I. Estados Unidos. III (3): 448-472.

1951*b*. II. Méjico. III (4): 555-570.

1952*a*. III. América Central. IV (1): 75-84.

1952*b*. IV. Colombia, Venezuela e Guaianas. IV (3): 255-280.

1952*c*. V. Ecuador y Perú. IV (4): 319-332.

1953*a*. VI. Bolivia e Paraguay. V (1): 11-16.

1953*b*. VII. Chile. V (2): 131-136.

1955*a*. VIII. Argentina. VII (1): 143-176.

Dias, E. — «Chagas disease», *World-Atlas of Epidemic Diseases*, part. II: 135-139 (con mapas), 1953*c*.

Dias, E. — «Variações mensais da incidência das formas evolutivas de *T. infestans* e do *P. megistus* no município de Bambuí, Minas Gerais», *Mem. I. O. Cruz*, LIII (2, 3, 4): 457-472, 1955*b*.

Dias, E. — «Observações sobre eliminação de dejecções e tempo de sucção em alguns triatomíneos sul-americanos», *Mem. I. O. Cruz*, LIV (1): 115-124, 1956.

Dias, E., y Torrealba, J. E. — «Infecção natural de *Eutriatoma maculata* pelo *Schizotrypanum cruzi* no Brasil e na Venezuela», *Mem. I. O. Cruz*, XXXIII (2): 249-252, 1938.

Di Primio, R. — «Novas investigações sobre a distribuição geográfica dos triatomíneos e de seus índices de infestação no Rio Grande do Sul», *Rev. Med. R. G. do Sul*, IX (52): 3-9, 1953*a*.

Di Primio, R. — «Sobre a *Triatoma rubrovaria* (Blanchard, 1843) no Rio Grande do Sul», *Rev. do R. G. do Sul*, IX (54): 5, 1953*b*.

Di Primio, R. — «Transmissores da doença de Chagas e respectivos índices de infecção no Rio Grande do Sul», *An. Fac. Med. Porto Alegre*, ano 12.°-13.° (separata), 1954*a*.

Di Primio, R. — «Pesquisa de triatomíneos no Alto Taquari e Montenegro, no estado do Rio Grande do Sul», *Rev. Med. R. G. do Sul*, X (60): 12, 1954*b*.

Di Primio, R. — «Distribuição geográfica de *Panstrongylus megistus* no Rio Grande do Sul», *Ann. Fac. Med. Porto Alegre*, ano 15 (fasc. único), 1955.

Faust, E. C. — «The etiologic agent of Chagas' disease in the United States», *Bol. Of. San. Pan.*, XXVIII (5): 455-461, 1949.

Espinoza, L. — «Epidemiología de la enfermedad de Chagas en el Ecuador», *Rev. Ecuat. Med. Trop. e Hig.*, XII (1): 25-106, 1955.

Cajardo Tobar, R., y Thiermann, E. — «Comprobación de la existencia de un nuevo tripanosoma en el país», *Bol. inf. Paras. Chile*, V (2): 23-25, 1950.

Cajardo Tobar, R. — «Capacidad de ayuno de triatomídeos chilenos», *Bol. Inf. Par. chilenas*, VII (4): 56-60, 1952.

Gasic, G. — «Algunos hechos sobre clínica y epidemiología de la enfermedad de Chagas en Chile», *Bol. Of. San. Pan.*, XXII (?): 327-335, 1943.

Gonzalez, G., Arce, Q. A., y Rivarola, J. B. — «Investigación sobre la enfermedad

de Chagas en el Chaco Paraguayo», *An. Fac. Cienc. Méd.*, x (16): 21-42, 1942.

HACK, W. H. — «Estudios sobre la biología de *Triatoma infestans* (Klug. 1834)», *An. Inst. Med. Reg. (Univ. Nac. Tucumán)*, IV (2): 125-148, 1955.

HERRER, A. — «Trypanosomiasis americana en el Perú. I. El insecto vector y los animales que actúan de reservorios de la enfermedad de Chagas en la región sudoccidental», *Rev. Med. Exp.*, IX: 23-27, 1955*a*.

HERRER, A. — «Trypanosomiasis americana en el Perú. III. Importancia del cobayo como reservorio de la enfermedad de Chagas en la región sudoccidental», *Rev. Med. Exp.*, 45-54, 1955*b*.

HERRER, A. — «Trypanosomiasis americana en el Perú. IV. Ingreso del *Triatoma infestans* al territorio peruano, su dispersión en éste y posibilidad de ser erradicado», *Rev. Med. Exp.*, IX: 57-65; 2 fotogr., 1955*c*.

HERRER, A. — «Trypanosomiasis americana en el Perú. V. Triatomíneos del valle interandino del Marañon», *Rev. Med. Exp.*, IX: 69-81; 2 fotog., 1955*d*.

HERRER, A. — «Observaciones sobre la enfermedad de Chagas en la provincia de Moyobamba (dpto. de Santander)», *Rev. Med. Exp.*, X: 59-73; 2 fotog., 1956.

IÑIGUEZ MONTENEGRO, C. — «Patología regional de una zona del norte de Córdoba, Sebastián Elcano», *Rev. Med. Córdoba*, XXXIV: 701-724, 1946.

IRIATE, D. — «Investigaciones parasitológicas y entomológicas sobre la enfermedad de Chagas», *Bol. Lab. Clin. L. Razetti*, I (3): 45-64, Venezuela, 1941.

JORG, M. A.— «Límite sur de la dispersión geográfica de *Triatoma infestans* y su infestación por *Trypanosoma cruzi* en la Argentina», *Bol. Of. San. Pan.*, XLII (1): 59-66; 1 mapa, 1957.

LENT, H. — «O género *Rhodnius* Stal, 1859. (Hem. Reduv.)», *Rev. Bras. Biol.*, VIII (3): 297-339, 1948.

LOBO, A. G. S., BORBA, A. M., y SOUSA, J. DE — «Contribuição ao conhecimento da distribuição dos triatomíneos domiciliários e seus índices de infecção natural pelo *S. cruzi* no estado de Paraná», *Rev. Bras. Mal. e Doenç. Trop.*, VI (4): 571-588, 1954.

LUCENA, T. DE — «Subsídio para o estudo epidemiológico da doença de Chagas no Nordeste», *Rev. Bras. Mal. Doenç. Trop.*, IV (2): 171-176, 1952.

LUCENA, T. DE, y COSTA, L. — «Estudos preliminares sobre a doença de Chagas na Paraíba», *Rev. Bras. Mal. Doenç. Trop.*, V (1): 55-68, 1953.

MACHADO, H., y SILVA PINTO, O. DA — «Contribuição ao conhecimento da distribuição geográfica dos triatomídeos domiciliários e de seus índices de infecção natural no estado de Ceará, Brasil», *Rev. Bras. Mal. Doenç. Trop.*, IV (2): 157-170, 1952.

MAYER, H. F., y ALCARAZ, I. L. — «Estudios relacionados con las fuentes de alimentación de *Triatoma infestans*», *An. Inst. Med. Reg. (Univ. Nac. Tucumán)*, IV (2): 195-201, 1955.

MAZZA, S. — *Comprobación de casos agudos de enfermedad de Chagas en nuevas partes de la zona biológica chaqueña (Formosa, Chaco salteño). Hallazgos epidemiológicos especiales de la región.* Mis. Est. Pat. Reg. Arg., publ. n.° 27; Buenos Aires, 1936.

MAZZA, S. — *Enfermedad de Chagas en las provincias de Salta y Jujuy.* Mis. Est. Pat. Reg. Arg., publ. n.° 45; Buenos Aires, 1940.

MAZZA, S., TALICE, R. V., y JORG, M. A. — *Investigaciones sobre Triatomidae. Sobre* T. (Neotriatoma) circummaculata *y* T. (Eutriatoma). Mis. Est. Pat. Reg. Arg., publ. n.° 62; Buenos Aires, 1942.

MIRANDA, C. — «Nota sobre os transmissores da moléstia de Chagas ocorrentes no estado de Maranhão, Brasil», *Mem. I. O. Cruz,* XXXIX (3): 297-300, 1943.

NEIVA, A. — «Informações sobre a biologia do *Conorhinus megistus* Burm», *Mem. I. O. Cruz,* II (2): 206-212, 1910.

NEIVA, A., y LENT, H. — «Sinopse dos triatomíneos», *Rev. Entomol.,* XII (1-2): 61-92; il.; Rio de Janeiro, 1941.

NEIVA, A., y PENNA, B. — «Viagem científica pelo Norte de Baía, Sudoeste de Pernambuco, Sul de Piauí e do norte a sul de Goiás», *Mem. I. O. Cruz,* VIII (3): 74-224; 1 mapa y fotog., 1916.

NIÑO, F. A. — *Contribución al estudio de la enfermedad de Chagas o tripanosomiasis americana en la República Argentina.* Tesis. Fac. Cienc. Med., Buenos Aires, 1929.

OSIMANI, J. J., VERISSIMO, S., y BAYLE CARBONELL, P. — «La profilaxia de la enfermedad de Chagas en el Uruguay por medio del gamexano: experiencias realizadas. Plan de lucha contra el *Triatoma infestans*», *Bol. Of. San. Pan.,* XXIX (11): 1125-1134, 1950.

PELLEGRINO, J. — «Distribuição e índice de infestação dos triatomídeos transmissores da doença de Chagas no Sudoeste de Minas Gerais», *Rev. Med. Bras.,* V (8): 55-56 (Ref. Sem. Chagas, 9/2/1956), 1948.

PEÑALVER, L. M., FAJARDO, J., y AGUILAR, F. J. — «Aportes al conocimiento de la enfermedad de Chagas en Guatemala», *Rev. Col. Med. Guatemala,* IV (1): 20-35, 1953.

PEREIRA DA SILVA, L. H., CARVALHO, S. S. DE, y CARNEIRO, M. N. R. — «Doença de Chagas na Paraíba. Inquérito serológico preliminar», *Rev. Bras. Mal. Doenç. Trop.,* VIII (1): 281-288, 1956.

PERLOWAGORA SZUMLEWICZ, A. — «Ciclo evolutivo do *Triatoma infestans* em condições de laboratório», *Rev. Bras. Mal. Doenç. Trop.,* V (1): 35-48, 1953.

PESSOA, S. B. — *Problemas Brasileiros de Higiene Rural* (cap. X: «Moléstia de Chagas»; pp. 303-336). São Paulo, Brasil, 1949.

PESSOA, S. B. — *Parasitologia Médica.* Ed. Guanabara, Rio de Janeiro. 3.ª ed., 885 pp., 465 figs., 1951.

PIFANO, F. — «La enfermedad de Chagas en Venezuela», *Bol. Of. San. Pan.,* IX (10): 984-988, 1940.

PINTO, C. — «Biologia de *Triatoma brasiliensis* Neiva», *Scienc. Med.,* ano II (10): 541-543, 1924.

PINTO, C. — *Artrópodos Parasitos e Transmissores de Doenças.* Ed. Pimento de Mello e C.ª, Rio de Janeiro, 1930.

PINTO, C. — «*Trypanosomiasis cruzi* (doença de Carlos Chagas) no Rio Grande do Sul», *Mem. I. O. Cruz,* XXXV (4): 443-538, 1942.

PONDE, A. — «Enfermedades parasitarias de importancia social. II. Miocarditis chagásica», *Congr. Pediatr. Montevideo,* I: 372-377, 1953.

PORTER, C. E. — «Importancia médica que adquiere un hemíptero heteróptero chileno», *Rev. Chil. H. N.,* XLII: 122-124, 1938.

SEGOVIA, J. C. — «Tripanosomiasis en El Salvador», *Arch. Hosp. Rosales, El Salvador,* XXXIV (80): 885-888, 1942.

SILVA PINTO, O. DA — «Contribuição ao conhecimento da distribuição geográfica dos triatomídeos domiciliários e de seus índices de infecção natural pelo *S. cruzi* no estado de São Paulo, Brasil», *Rev. Mal. Bras. Doenç. Trop.,* IV (2): 131-144, 1952.

SILVA PINTO, O. DA, BIGALHO, C. J., y GUEDES, A. S. — «Contribuição ao conhecimento dos triatomídeos e de seus índices de infecção natural pelo *S. cruzi* no estado de Minas Gerais, Brasil», *Rev. Bras. Mal. Doenç. Trop.,* IV (2): 105-130, 1952.

SILVA, T. L. DA, y CORRÊA, R. R. — «Informes sobre a distribuição dos triatomíneos na área paulista», *Fol. Clin. Biol.,* XXII (1-2): 79-84, 1954.

SIMÕES, A. J. P. — «Doença de Chagas no estado de Paraná, Brasil. Esboço epidemiológico», *Mem. I. O. Cruz,* XXXIX (3): 279-290, 1943.

SOUSA ARAÚJO, H. C. DE — «A doença de Chagas no Paraná», *Mem. I. O. Cruz,* LII (2): 477-485, 1954.

TALICE, R. V., COSTA, R. S., RIAL, B., y OSIMANI, J. J. — «Enfermedad de Chagas (tripanosomiasis americana)», *Mem. Inst. Hig. de Montevideo,* 349, pp., il., 1940.

TALICE, R. V., y OSIMANI, J. J. — «Primer caso de infección natural del perro por *Trypanosoma cruzi*», *An. Fac. Med. Montevideo,* XII: 6, 1937.

TALICE, R. V., VERISSIMO, S., OSIMANI, J. J., y FRANCA, M. E. — «Estudio epidemiológico sobre la enfermedad de Chagas en la zona endémica del Uruguay», *Bol. Of. San. Pan.,* XXXIII (6): 595-620, 1952.

TORANZOS, L. B. — «La infestación del *Triatoma (Eutriatoma) rubrovaria* por el *Schizotrypanum cruzi.* Su comprobación en la República Argentina», *An. Inst. Med. Regional (Univ. Nac. Tucumán),* I (1): 105-116, 3 figs., 1944.

TORREALBA, J. F. — «Pequeñas observaciones sobre el *Rhodnius prolixus* y tripanosomiasis en el distrito Zaragua (Guárico)», *Gac. Méd. Caracas,* XL (13): 179-181 (y 1943: CVI; 19-25), 1953.

TORRICO, R. A. — «Conocimientos actuales sobre la epidemiología de la enfermedad de Chagas en Bolivia», *Bol. Of. San. Pan.,* XXIX (8): 827-840, 1950.

ZELEDÓN, A. R. — *El problema de la tripanosomiasis americana o enfermedad de Chagas en Costa Rica.* Tesis de Grado. Univ. Costa Rica, Fac. de Ciencias. (Min. Salubr. Publ., Dir. Gen. Salubr. Investig. Epidemiológicas, n.° 2), 109 pp., il., 1952.

# MEDICAL CARTOGRAPHY AS A SCIENTIFIC METHOD FOR THE INVESTIGATION OF TROPICAL DISEASES

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The task of medical cartography is to record by means of medical maps observations concerning the occurrence of diseases and changes with regard to time and place in this occurrence. A medical map not only depicts the topography of a part of the earth's surface, but also presents medical facts in a clearly arranged graphical manner. By means of such a map it is possible to read off and critically compare the incidence of a disease in a given region and its changes with regard to both time and location. The emphasis is placed not upon the clinical description of the disease, but rather upon the nosologic union of the nature of a disease and its causative agent. Consideration of the different intensities of occurrence of a disease on a medical map permits the area of predilection to be recognized. This type of record has made possible the first medical-geographic classification of the diseases of mankind — as an ubiquitous dweller of the earth. We can differentiate:

*A*) Ubiquitously occurring diseases, e.g., tuberculosis;
*B*) Zonally restricted diseases, e.g., sleeping sickness, yellow fever;
*C*) Locally limited diseases, e.g., schistosomiasis, tick-borne and mite-borne typhus.

A differentiation between native (= indigenous) and exotic (= tropic) diseases has long been customary in the speech usage of physicians in the more temperate zones. But only lately has due regard been given to the question of the origin of this difference

in the distribution of tropical and non-tropical diseases. In the collective term «tropical diseases» there is already an expression of the relationship of diseases of this group to geographic and climatic conditions. The term «tropical» may be considered in this case not only of geographic, but also probably of biologic significance.

This double meaning is particularly well expressed in the term «zone without thermic seasons», which the German geographer Prof. Dr. Carl Troll has coined. Prof. Troll says: «The absence of thermic seasons is a characteristic of all climates of the inner tropics from lowlands to mountain regions.» He further states: «in the tropics the seasons are in general determined from precipitation, atmospheric humidity and water economy.» Therefore, there are particular conditions of temperature and humidity, which enter into the particular nosologic conditions in the tropics. For example: *diseases of group A* with ubiquitous occurrence (typhoyd fever, diphtheria, scarlatina), which show a marked seasonal rhythm in the temperate zones, occur almost equally distributed in the individual months of the year in those regions which belong to the «zone without thermic seasons» — or there may be another type of seasonal rhythm which corresponds to the change between seasons of dry and rainy weather (e.g., cerebrospinal meningitis in Africa).

*The diseases of group B and C* with zonal and local occurrence in the tropics also show certain relationship to geographic and climatic factors. Further investigation can be made with the help of medical cartography. Prerequisite is an exact knowledge of the distribution of the individual tropical diseases, e. g., a cartographic representation of the historical and present situation. This can be best carried out by use of physical maps with colored markings of the epidemic and endemic autochthonous occurrence of each individual disease.

The cartographic representation of a tropical disease must yield the following information:

1) The area of predilection;
2) The potential area of occurrence;
3) The borders of these areas.

The task of the geomedical research of tropical diseases consists in explaining, especially by means of medical maps, the correlation between a disease and the natural factors of the physical environment,

including the climate, vegetation, fauna with the animal vectors. Its task also consists in explaining the correlation between a disease and the cultural and sociological conditions of the native population of the infested area.

The task of medical cartography is to give in a map as simple a survey as possible of the biological and ecological connections between the occurrence of a disease and the geographic and climatic conditions of the region.

In the cartographic representation there are the following important differences in tropical diseases:

1) Diseases which must naturally remain limited to the tropics, on account of the limitation of the ecological relations of their vectors, e.g., leishmaniasis, dengue, etc.;

2) Diseases which are artificially restricted to the tropics, because they have already been eliminated in the countries of the temperate zones by application of specific prophylactic measures or through the general level of hygiene, e.g., smallpox, plague, etc.

However, the tropics still remain a reservoir for these diseases, out of which importation into the countries of the temperate zones can result at any time. Therefore, medical maps of these tropical reservoirs are necessary. These maps have value as documentation.

It will be necessary, from time to time, to renew this documentation of this «panorama of tropical diseases». The Geomedical Research Unit of the Heidelberg Academy of Sciences therefore intends to reissue from time to time new medical maps with a current representation of the most important tropical diseases. This will be done as a supplement to the maps of the *World Atlas of Epidemic Diseases*. In this manner, it will be possible to make a continuous cartographic registration of the distribution of the most important tropical diseases. These maps represent both important documentation of the necessity of control measures and also of their success.

I would like to take this opportunity of thanking all the scientists who have supported us in the presentation of the maps by sending data and who have helped us by answering our questions. I would also like to express the hope that all officials and scientists will be so kind as to assist us further in the preparation of new maps.

EXAMPLES

I. — There are different possibilities for characterising the area of distribution of zonal or local diseases: e.g., boundary lines of climatic character can be used as boundary lines for the distribution of a disease.

Isotherms, which can be deduced from the ecology of the vector, can be used as boundaries of a potential disease area.

For the occurrence of dengue fever in Europe the northern boundary is drawn by the annual isotherm of 55°F = 12.8°C, by which the Mediterranean climate is bounded.

II. — One can assume that the northern boundary line for the occurrence of autochthonous leishmaniasis in Europe is the isotherm of the annual average temperature of 10°C, because the vector of leishmaniae, the sand fly, can not reproduce north of this line.

III. — Also certain formations of vegetation can be entered in the medical maps and thus the area of predilection of a tropical disease can be brought out.

The area of tropical rain forest and other forest formations in Africa and South America may be used for the representation of occurrence of yellow fever there, because the monkeys living in them can be regarded as a reservoir for yellow fever virus.

# THE GEOGRAPHY OF CHAGAS DISEASE

ERNEST RODENWALDT

In his well known cartographic representation of the incidence of Chagas disease and its vectors, published in the *World Atlas of Epidemic Diseases,* Emmanuel Dias frankly admits that the geographical distribution of this disease has as yet not been fully elucidated. Although Dias' maps offer a true picture of the main areas of incidence, they depict merely the regions where the most intense investigations have been carried out. Future investigations may be expected to reveal a much wider spread of the condition. The same holds true for the picture of the incidence of the transmitting reduviidae, especially with regard to the degree of natural infection in some of those genera and species in which *Schistosoma cruzi* has been found.

Nevertheless, these points are counterbalanced by other facts which allow us to attribute a high degree of reliability to the cartographic representation of Chagas disease. In most parts of South and Central America the areas of incidence of the disease coincide with the degree of population density. This shows most clearly in all regions infested with Chagas disease located between the tropics, i.e., for Guatemala, San Salvador, Costa Rica, Panama, Venezuela, Columbia, and Ecuador, as well as for Brazil north of the tropic of Capricorn. Likewise, this holds true for the Argentine south of 41° latitude, and Chile south of 35° latitude. The absence of Chagas disease in the vast reaches of the Amazonas River and its tributaries, as well as in those of the Orinoco River, may be attributed to the fact that these areas are practically non-inhabited. Similarly the Argentine provinces south of the Negro River are very sparsely settled or even non-inhabited. Consequently — if I may anticipate it here — it appears rather doubtful that the coincidence of the southern border of the

incidence of Chagas disease and the 25° January isotherme should be of any significance.

Nevertheless, there are exceptions to the positive correlation between incidence of the disease and population density. South of the tropic of Capricorn the situation may even be reversed to a negative correlation. Uruguay, with its sparse population, shows areas of heavy incidence, while the neighbouring more densily populated Argentine provinces are free of the disease, despite a similar geographical situation. On the other hand, the sparsely settled northern provinces of the Argentine show a high incidence.

This difference in incidence in areas south of the tropic as against north of the tropic must, therefore, be attributed to factors other than population density.

In the United States, naturally infected triatomes, but no human infection, have been detected up to 37° north latitude.

Dias has pointed out that undoubtedly a very important, if not the decisive factor for the incidence of the disease is the social situation of the population and particularly their condition of living in crowded quarters, as they are found in most rural regions where primitive mud huts with thatched roofs and mats offer favourable breeding places to the triatomes. This in combination with a dense population constitutes increased health hazards.

The current worldwide tendency of accumulation of people in big cities conditions that large crowds reside at the fringes as well as in the centers of the towns in substandard dwellings, such as the ranchos in Venezuela, or the Brazilian favelas. If this development is going to continue, the hazards of the spread of the disease will increase and will require strict countermeasures. It is not the population density as such, but the congestion and mode of dwelling of a primitive and ignorant population, which may have the most unfavourable effects, even in less congested settlements. Mention should be made of the fact that, within these settlements, the various age and sex groups are equally exposed to infection. Also differences in race are of no consequence.

However, population density and type of settlement are not the only decisive factors in the incidence of the disease since there exists no direct transmission from one person to another. They represent,

however, the most important precondition for the transmission of *Schisotrypanum cruzi* by the reduviidae.

Chagas' discoveries gave rise to wide-range entomological research, leading to a very precise knowledge of the American reduviidae. The most essential result was the finding that the role of vector is being played by the following three species: *Triatoma infestans, Panstrongylus megistus,* and *Rhodnius prolixus,* out of the known genera of triatominae *Triatoma, Panstrongylus* and *Rhodnius.* This is due to the fact that these three species have adapted themselves to human dwellings, which have become their biotope. Precipitation tests have shown that these three species predominantly feed on human blood.

Therefore, it may *a priori* be assumed that areas with a prevalence of one or more of these species are highly susceptible for the spread of Chagas disease, increasingly so if a low living standard coincides with a high population density.

In Central America as well as in the northern territories of South America, i.e., in Guatemala, San Salvador, Costa Rica, Panama, Venezuela, and Columbia, *Tr. dimidiata* and *Rhodnius prolixus* have been found at the same time, together with a species of the genus *Eratyrus. Rhodnius prolixus* is found only between the tropics, where in combination with *Tr. infestans* it constitutes a double danger for these areas. Probably *Rhodnius prolixus* represents the main vector, as will be shown later in connection with a special biological characteristic.

A similar double hazard is found in Brazil north of the tropic, where a dense population at low living standard is exposed to the double attack of *Tr. infestans* and *Panstrongylus megistus.* The high incidence of the disease depicted on the map represents a reality and is not merely a sign that these areas have been more carefully investigated.

South of the tropic, however, *Rhodnius prolixus* does not occur, and *Panstrongylus megistus* is far less common than *Tr. infestans.* It would seem possible that the incidence of Chagas disease in Uruguay, Chile and the Argentine is to be attributed exclusively to *Tr. infestans.*

This obvious discrepancy in the correlation between population density and spread of the disease in the territories north and south

of the tropics seems to suggest that *Rhodnius prolixus* may be held responsible for the spread in Central America and in the northern parts of South America, whereas *Panstrongylus megistus* plays this role in Brazil north of the tropic.

In addition to the three aforementioned species of triatomes being considered as the dangerous ones, other genera and species have been found naturally infected with *Schizotrypanum cruzi*. This infection may take place via numerous mammals, but also via birds and cold-blooded animals carrying the parasite. All triatomes are considered as being potentially infective. Epidemiologically, however, they are insignificant as vector to man, except for those species living in human dwellings or living in a semidomestic state. They have no contact with human beings. Where the aforementioned three species, living and breeding in human habitations, establish contact, they become far more dangerous by their occurrence in large quantities in a highly congested human population.

Just as with malaria, where a relatively small number of anopheles have been recognized as transmitters, investigation of the biological characteristics of the triatomes is essential for conducting countermeasures. As we know, only the knowledge of specific biological features of certain anopheles has made it possible to conduct a special course of attack.

The development cycle of the house-dwelling triatomes is known in detail. It seems noteworthy, in view of their danger, that *Rhodnius prolixus* reproduces twice per year. Other species produce one new generation a year, another species produces a new generation in a two-year period. This factor must be of great consequence to the density of the vector population, since both larvae and adults of either sex feed on human blood and carry the parasite.

It may be assumed that the insects are generally spread by passive transport rather than by own flight. Nontheless it would be of interest to determine the flight range of the various species so as to avoid deviation.

It was Chagas who indicated that the disease occurs mainly during the warm months. This suggests an optimum temperature of 20°C to 30°C and shows the importance of environmental factors for the epidemiology of the disease.

Among the geographical factors, the elevation is apparently insignificant. *Triatoma infestans* has been found up to 4,000 m altitude, while others were detected at only a few hundred meters height. It would be of interest to know more about the behaviour of the most important species in this respect. It is of epidemiological significance that *Tr. infestans* avoids coastal areas on the Pacific as well as on the Atlantic coast of South America, while other species, such as *Tr. dimidata,* are found on the Pacific coast and are not found on the Atlantic coast. This fact may be attributed to climatic conditions, especially humidity factors. In general, areas of medium elevation are preferred to low coastal regions. This seems to indicate that a medium air humidity level in combination with medium temperatures is preferable to the insects. A high degree of humidity is not tolerated by some species.

A dry humid climate with average temperatures with annual fluctuations of 14°C and with a relative humidity of 45-70 percent is considered as an optimum, at least for certain species. Dry areas in the Anden mountain ranges are considered unfit for triatomes (this applies probably to *Tr. infestans* only), just as are low lying cool coastal areas. Considering the fact that certain insects, as for example anopheles, require optimal conditions within a very limited range, it seems imperative to study most carefully these limits.

Dias' very impressive maps show that in northern Brazil the local triatomes prefer low lying areas with a low degree of precipitation to areas with abundant rains. In this respect much is left to be done for research. Although any control of the climatic conditions is beyond human reach, it may be a determining factor in the selection of certain areas when new settlements are to be founded.

# DISTRIBUTION GÉOGRAPHIQUE DES TRIATOMIDÉES DANS LA VALLÉE DU SÃO FRANCISCO ET SES RAPPORTS AVEC LES CONDITIONS CLIMATIQUES [1]

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## CONSIDÉRATIONS GÉNÉRALES

Le travail le plus récent sur les aspects climatiques de la distribution des transmetteurs de la maladie de Chagas en territoire brésilien est celui présenté par Emmanuel Dias et Salomão Serebrenick au XVIII[e] Congrès International de Géographie, qui s'est tenu à Rio de Janeiro, en août 1956 [2].

Ce travail se rapportait à une vaste région centrale du Brésil — la Vallée du São Francisco — qui couvre plus de 600.000 km² et comprend une variété raisonnable de climats: tempérés et tropicaux, secs et humides, littoraux et continentaux, de plaine et de montagne.

Cependant, les données de base utilisées dans ce travail étaient, à un certain point, insuffisantes: elles avaient en effet été extraites de la littérature éparse existant jusqu'alors sur le sujet.

Pour cette raison, la Commission de la Vallée du São Francisco, dans le cadre de son programme résolu d'assainissement général de

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[1] Ont collaboré à l'orientation et l'exécution du relevé de base: Emmanuel Dias (de l'Institut Oswaldo Cruz) et João Leite Ferreira Filho (de la Commission de la Vallée du São Francisco).

[2] E. Dias et S. Serebrenick, «The Transmitters of Chagas' Disease in the São Francisco Valley, Brazil».

la région, et en vertu de l'importance particulière qu'elle attribue à la campagne d'extermination des insectes responsables de la transmission de l'endémie squizotripanosique, décida de compléter la copie des données existantes, et procéda pour cela, en 1957, à un relevé systématique des triatomidées, couvrant pratiquement toute la région sous sa juridiction.

Ce fut un travail difficile, étant donné l'immensité de la région étudiée, en grande partie inhospitalière, lequel exigea un remarquable esprit de sacrifice de l'équipe des enquêteurs amenés à utiliser les moyens de transport les plus divers: avion, jeep, omnibus, canoe et cheval. Ce fut cependant une mission «payante», dont les résultats sont venus jeter la lumière sur le problème de distribution et le comportement des vecteurs de la maladie de Chagas, permettant ainsi une meilleure planification des grandes mesures à prendre pour son extinction.

## DISTRIBUTION DES TRIATOMIDÉES

L'utilité de cette nouvelle enquête ressort immédiatement de l'examen comparatif des cartes 1 et 2, sur la première desquelles on voit la distribution des vecteurs de la maladie de Chagas sur la base des données disponibles jusqu'en juin 1956, tandis que la seconde présente cette distribution sur la base des données déjà complétées des éléments spécialement collectés par la Commission de la Vallée du São Francisco, fin 1957.

Il se trouve ainsi vérifié que l'assertion antérieure selon laquelle aussi bien la fréquence que le degré d'infection des transmetteurs diminuaient vers l'aval, au point de se trouver négligeables dans le Bas São Francisco, entre Paulo Afonso et le débouché dans l'Océan, devait être rectifiée, étant donné que, dans ce tronçon inférieur — à l'exception d'une étroite bande littorale — on a trouvé des triatomidées en grande quantité et avec un indice élevé d'infection.

Ce nouveau panorama de distribution des «barbeiros» est notoirement plus sombre, car: alors qu'auparavant les triatomidées étaient considérées comme présentes dans seulement 72 pour cent des municipes de la Vallée du São Francisco, maintenant leur absence ne fut

constatée qu'en seulement 13 des 250 municipes existant dans la Vallée, soit pour seulement 5 pour cent de ceux-ci, ces rares municipes non atteints étant localisés quasi exclusivement dans la bande littorale; ainsi, donc, la présence des triatomidées en 95 pour cent

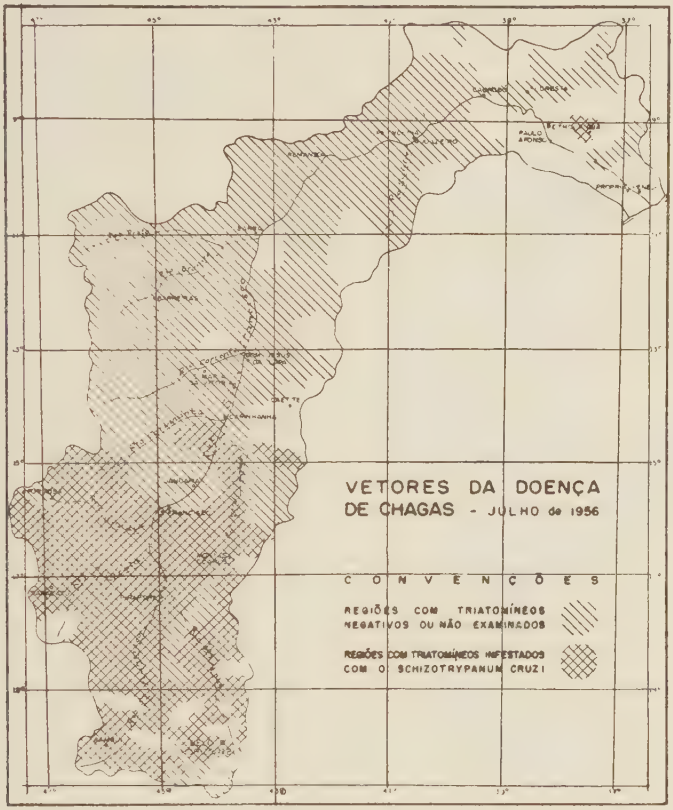


Fig. 1

des municipes de la Vallée autorise, sans exagération, à considérer la région comme entièrement couverte par l'existence des transmetteurs de la maladie de Chagas.

On obtint également de nouvelles indications individuelles sur la distribution et le degré d'infection des diverses espèces transmetteuses du *Schizotrypanum cruzi*.

Les espèces existantes les plus importantes dans la région sont au nombre de 5: *Panstrongylus megistus, Triatoma sordida, Triatoma maculata, Triatoma brasiliensis* et *Triatoma infestans.*

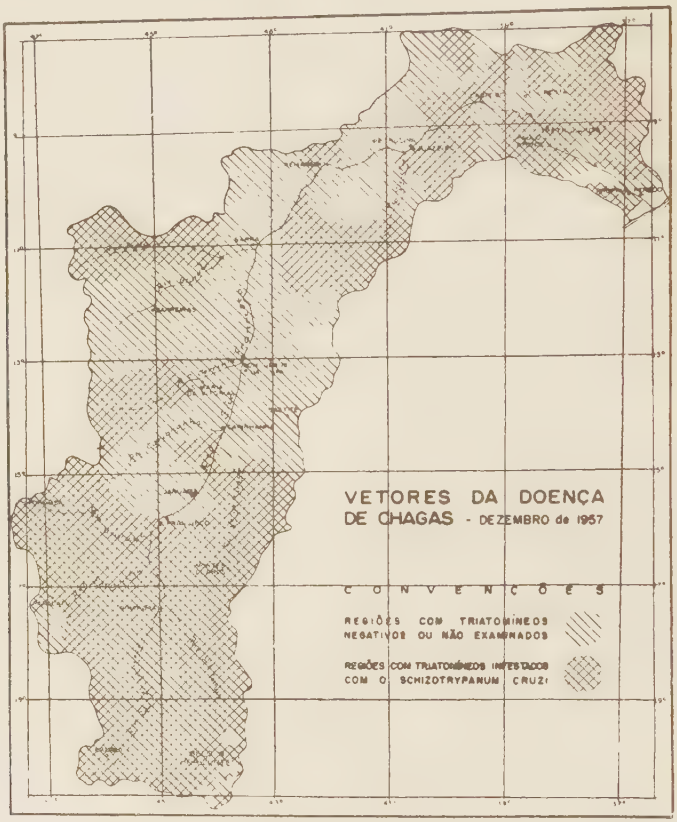


Fig. 2

Selon les données disponibles en mi-56, la présence de ces 5 espèces dans la Vallée s'exprimait par les chiffres suivants:

*Panstrongylus megistus* . . présent en 54 pour cent des municipes
*Triatoma sordida* . . . . . présent en 37 pour cent des municipes
*Triatoma infestans* . . . . présent en 13 pour cent des municipes
*Triatoma brasiliensis* . . . présent en 10 pour cent des municipes
*Triatoma maculata* . . . . présent en 7 pour cent des municipes

tandis que, au vu des données actualisées par le récent relevé, la présence de ces triatomidées se traduit de la façon suivante:

*Panstrongylus megistus* . . présent en 64 pour cent des municipes
*Triatoma sordida*. . . . . présent en 38 pour cent des municipes
*Triatoma infestans* . . . . présent en 13 pour cent des municipes
*Triatoma brasiliensis* . . . présent en 16 pour cent des municipes
*Triatoma maculata* . . . . présent en 18 pour cent des municipes

Ceci signifie que le développement sus-mentionné de la zone d'influence des triatomidées dans la Vallée concerne surtout les espèces *Triatoma maculata* et *Panstrongylus megistus* et, pour une bonne part, également le *Triatoma brasiliensis*.

Il reste pourtant frappant qu'une grande inégalité d'incidence subsiste entre les différentes espèces — parmi lesquelles se détache le *Panstrongylus megistus* —, quoique le critère adopté pour estimer cette influence apparaisse jusqu'à un certain point précaire, soit à cause de l'inégalité territoriale des municipes, soit parce qu'il n'est pas tenu compte du degré de dispersion à l'intérieur de chaque municipe.

Quant à l'indice d'infection des triatomidées, le récent relevé a révélé qu'il est plus grand pour les espèces *megistus* et *infestans*, comme on peut le voir sur le tableau suivant:

*Panstrongylus megistus* . . infecté dans 46 pour cent des municipes
*Triatoma sordida*. . . . . infecté dans 12 pour cent des municipes
*Triatoma infestans* . . . . infecté dans 7 pour cent des municipes
*Triatoma brasiliensis* . . . infecté dans 6 pour cent des municipes
*Triatoma maculata* . . . . infecté dans 3 pour cent des municipes

En ce qui concerne la variation dans l'espace de l'indice d'infection — sans considération des espèces de triatomidées —, on peut vérifier que cet indice est de l'ordre de 45 pour cent dans le Haut São Francisco (entre les latitudes 21° et 18°), de 50 pour cent dans le Haut-Moyen São Francisco (entre les latitudes 18° et 14°), de 10 pour cent dans la partie centrale du Moyen São Francisco (entre les latitudes 14° et 11°), de 25 pour cent dans la partie inférieure du Moyen São Francisco (entre les longitudes 43° et 38°) et de

50 pour cent dans le Bas São Francisco (à l'est du méridien 38°, exceptée la bande littorale).

Comme on a pu le constater ci-dessus, la distribution géographique des triatomidées dans la Vallée, basée sur le critère de sa pré-

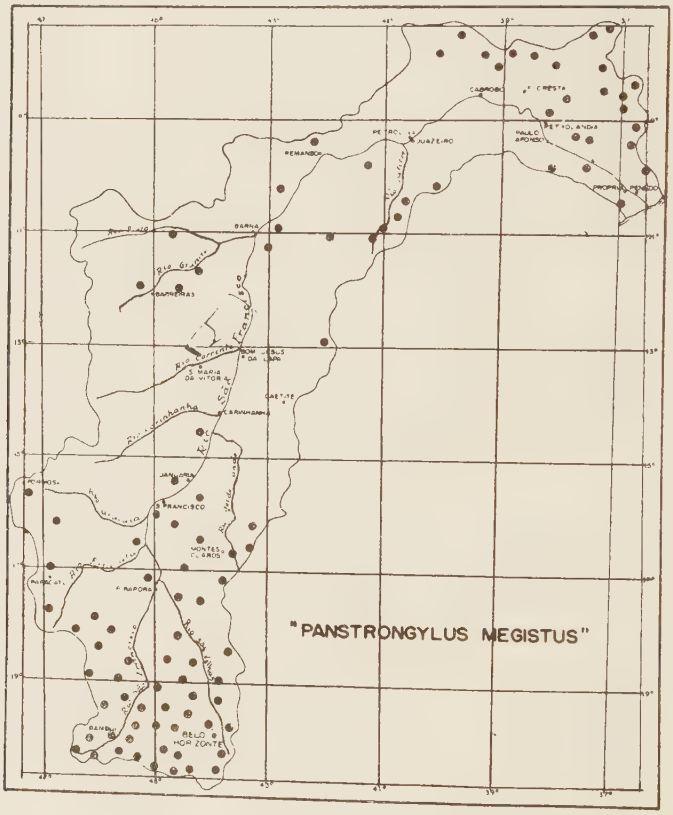


Fig. 3

sence dans les municipes, se révèle être quelque chose d'amorphe, signifiant seulement que 95 pour cent des municipes de la région accusent une existence de ces transmetteurs, sans que l'on puisse avoir cependant une idée de la fréquence spatiale, ou, autrement dit, de la densité à l'intérieur de chaque municipe, quoique le Haut

São Francisco, où les municipes sont de plus faible extension, se présente comme une région de forte concentration des «barbeiros».

Cependant, quand on en vient à considérer individuellement les espèces des transmetteurs, il en résulte des panoramas plus expressifs de leur distribution dans l'espace.

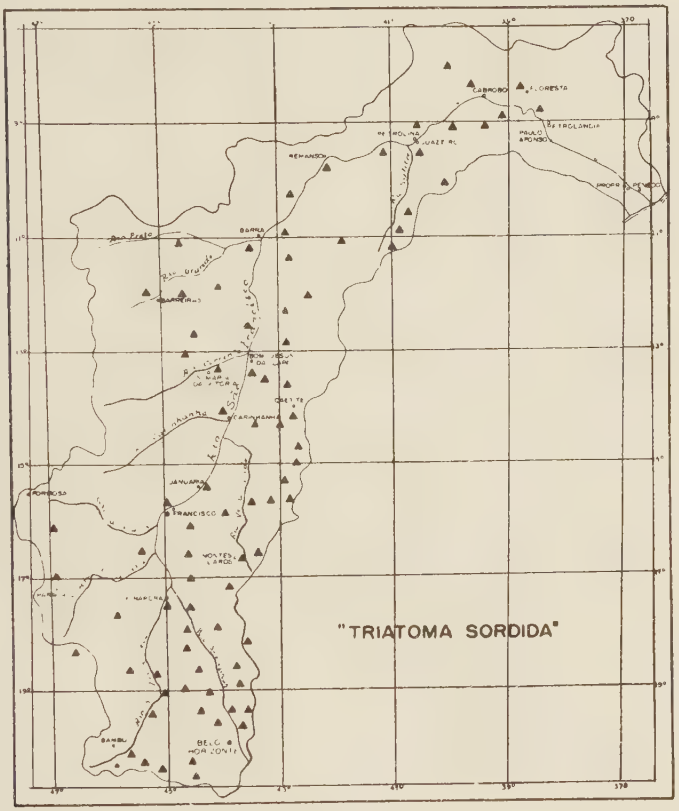


Fig. 4

Ainsi, on peut vérifier sur les cartes 3 à 7 que le *P. megistus* et le *T. sordida* sont les deux espèces les plus «cosmopolites» de la Vallée, la première présentant seulement deux vides, entre Carinhanha et Paratinga (14° à 12° de latitude) et entre Juazeiro et Cabrobó

(41° à 39° de longitude), et la seconde seulement un manque appréciable de Petrolândia à l'aval.

Déjà les trois autres espèces restantes montrent une nette prédisposition pour de plus petites régions déterminées de la Vallée, le

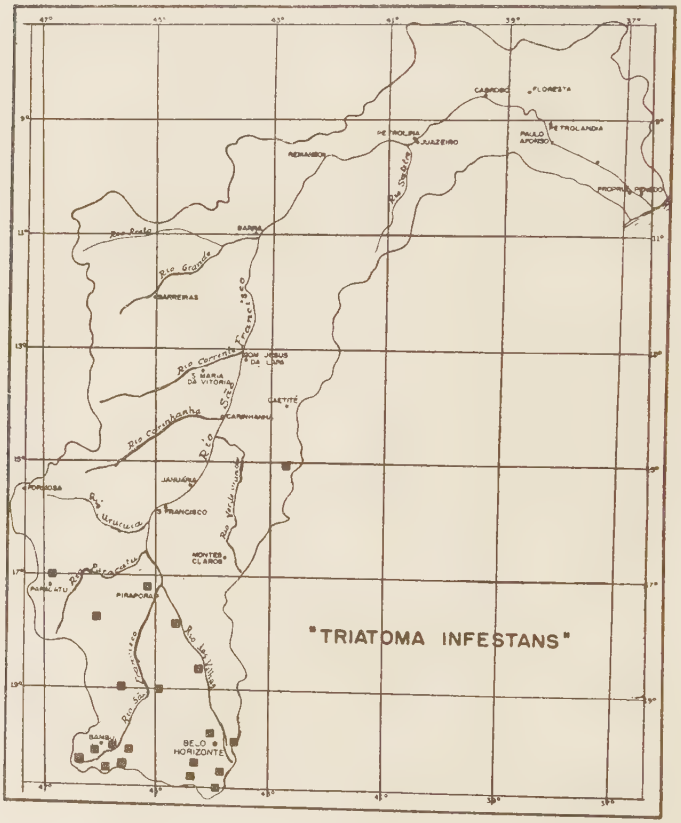


Fig. 5

*T. infestans* se limitant exclusivement à la partie méridionale du Haut São Francisco (au Sud du parallèle 17°), tandis que le *T. maculata* et le *T. brasiliensis* se localisent dans le Bas Moyen et le Bas São Francisco (à l'aval de Remanso).

INFLUENCES CLIMATIQUES

Bien que les données de base déjà disponibles actuellement soient raisonnablement suffisantes, il se révèle encore difficile d'interpréter

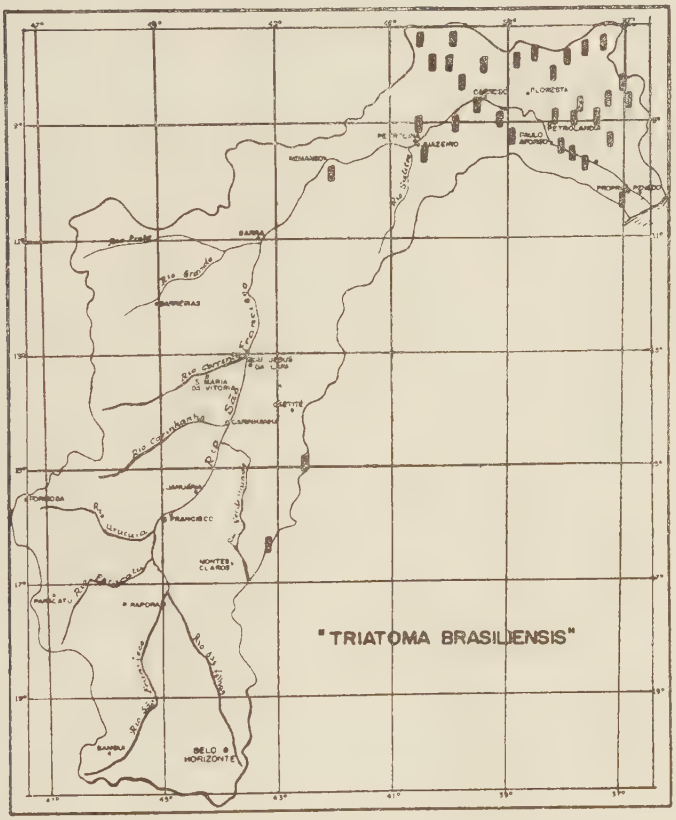


Fig. 6

avec sécurité l'influence éventuelle des conditions climatiques sur la prolifération et la distribution des «barbeiros» dans la Vallée du São Francisco.

La difficulté apparaît d'abord quand on constate que les aspects climatiques des différentes parties de la Vallée sont assez diversifiés et que, malgré cela, on trouve des «barbeiros» sur la quasi-totalité de son étendue. Il s'ensuit, effectivement, que, à l'inté-

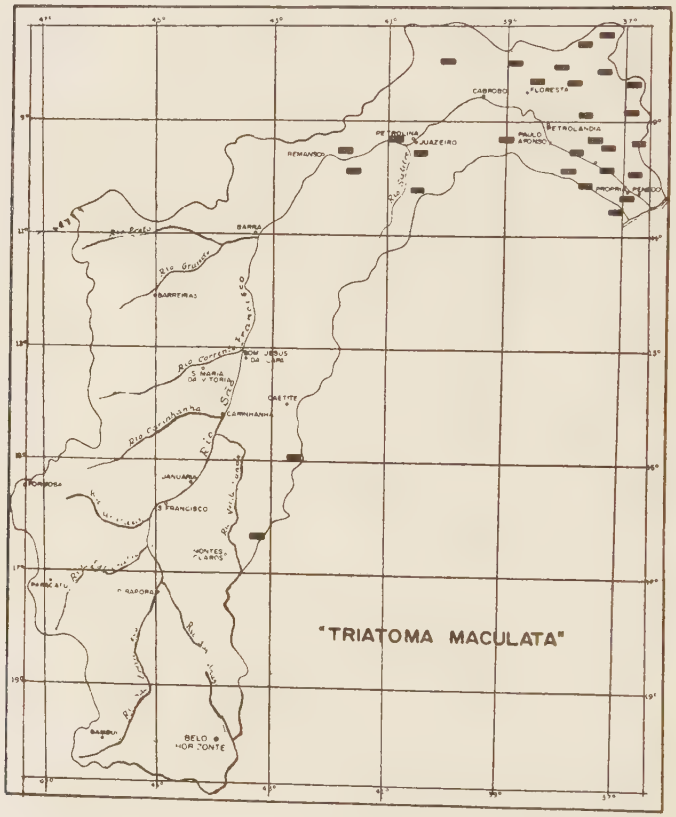


Fig. 7

rieur de la Vallée, les éléments météorologiques — isolés ou combinés — ne présentent pas de valeurs critiques déterminées par la physiologie des insectes en question, considérés dans leur ensemble.

Il reste uniquement, comme tentation de recherche, l'étroite bande littorale, de seulement 30 km à 40 km, où, comme on l'a vu plus

haut, n'ont été rencontrés jusqu'aujourd'hui de «barbeiros» d'aucune espèce.

Le fait d'établir une corrélation entre l'absence de «barbeiros» dans cette bande et une influence climatique quelconque — question

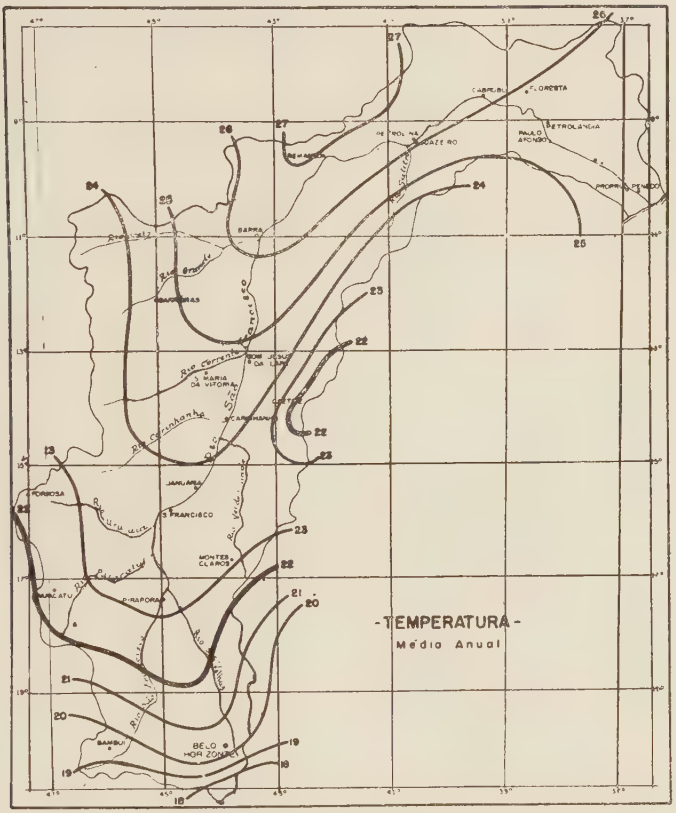


Fig. 8

qui sera reprise plus loin — ne paraît pas non plus tâche aisée, du fait que les valeurs isolées et même combinées des éléments climatiques dans cette bande ne diffèrent pas de celles rencontrées dans quelqu'autre point de la Vallée, où apparaissent pourtant des triatomidées.

S'il est difficile, comme nous l'avons montré, de déceler, de façon convaincante, une quelconque influence des conditions climatiques sur *l'ensemble* des espèces «barbeiros», il est cependant possible d'observer une certaine corrélation si on considère ces espèces *séparément*.

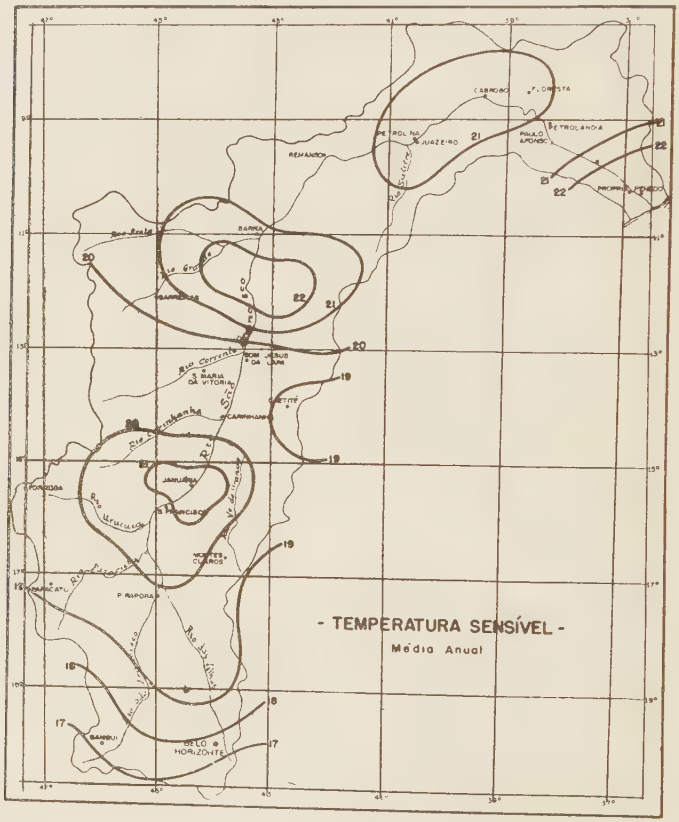


Fig. 9

Le cas le plus net de dépendance est suggéré par l'examen comparatif de la carte de distribution du *Triatoma infestans* (fig. 5) et des cartes climatiques (figs. 8 à 12).

Il apparaît que les caractéristiques de la région où l'on rencontre des *infestans* se traduisent ainsi:

*Température* moyenne annuelle inférieure à 23° (18° à 23°);
*Température humide* moyenne annuelle inférieure à 19° (16° à 19°);
*Humidité relative* annuelle supérieure à 73 pour cent (73 à 80 pour cent);
*Vent* moyen annuel inférieur à 2 m/s.

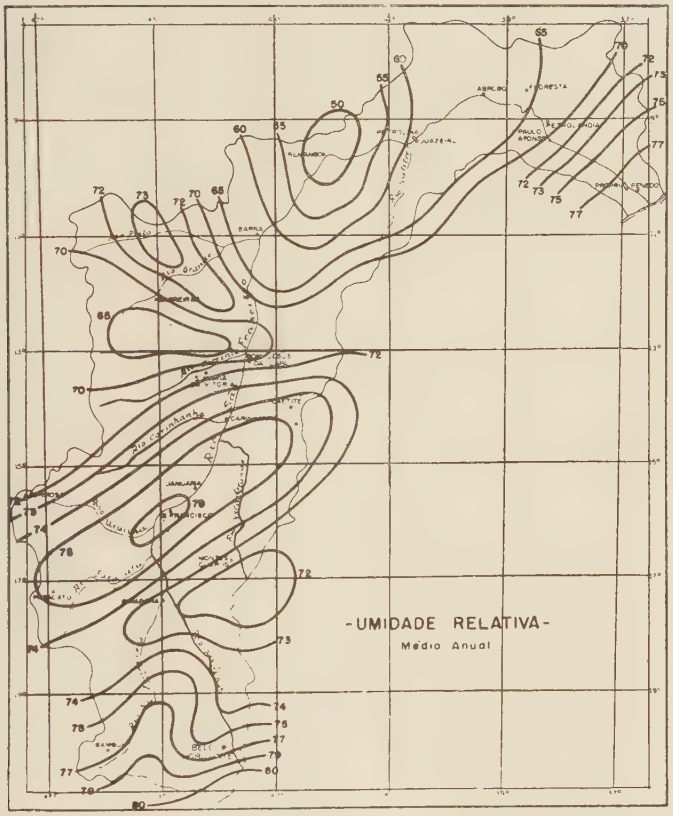


Fig. 10

outre d'autres données secondaires:

*Nébulosité* moyenne annuelle, 6/10;
*Pluviométrie:* total annuel, 1.100 mm à 1.900 mm.
*Évaporation* totale annuelle inférieure à 1.000 mm.

À l'exception de la *température*, la limite de rencontre du *T. infestans* ne suit aucune des isolignes des éléments ci-dessus, et les valeurs caractéristiques de la région se répètent dans d'autres points de la Vallée.

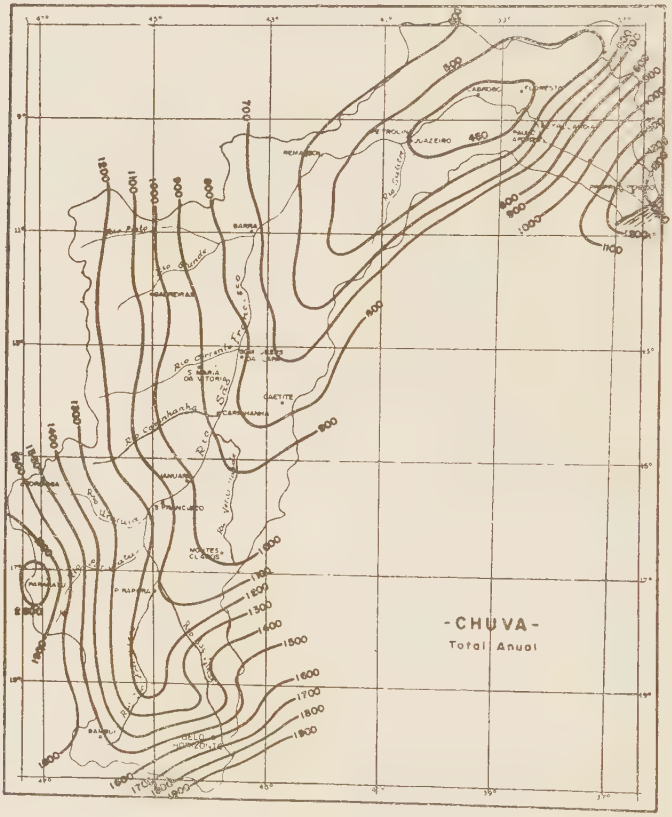


Fig. 11

On est autorisé, de ce fait, à tirer seulement une déduction unique: *le «Triatoma infestans» ne se rencontre pas avec des températures moyennes annuelles supérieures à 23°* (ou température humide supérieure à 19°).

Pour le *Panstrongylus megistus* et le *Triatoma sordida* — espèces cosmopolites qui sont disséminées dans la plus grande partie de la Vallée — on ne retire aucune conclusion acceptable. On peut, au

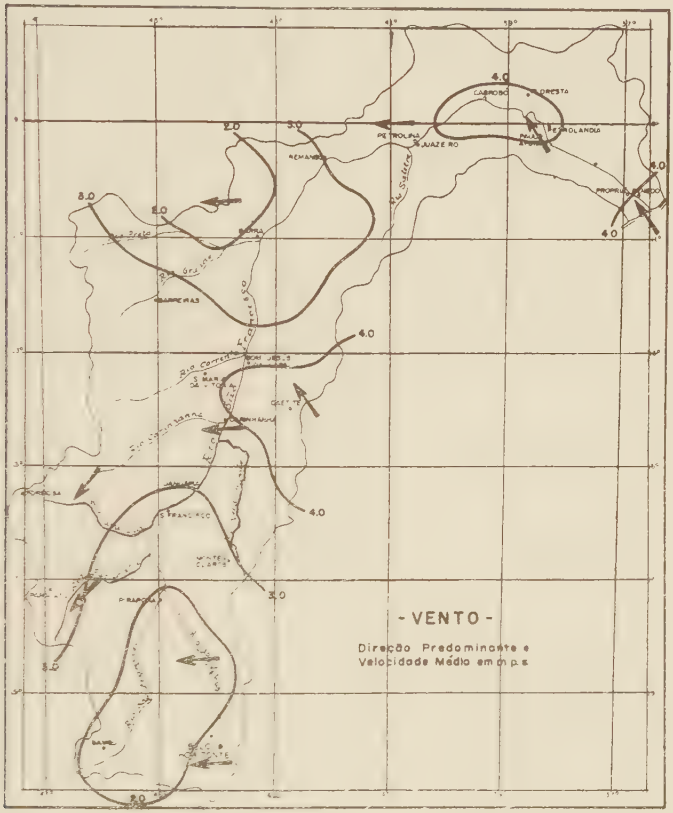


Fig. 12

plus, noter que, *dans les régions exemptes de «P. megistus»* (fig. 3), *le vent souffle fort* (plus de 4 m/s. en moyenne annuelle) (fig. 12), et que, *dans la région exempte de «T. sordida»* (fig. 4) *existe une combinaison température élevée* (25° à 26°) (fig. 8) et *humidité également relativement importante* (70 à 77 pour cent) (fig. 10).

Finalement, le *T. brasiliensis* et le *T. maculata* s'établissent de préférence en région chaude et semi-humide ou semi-aride, avec:

*Température* moyenne annuelle entre 25° et 27°;
*Humidité relative* annuelle entre 50 et 73 pour cent;
*Vent* moyen annuel fort, de 3 m à 4 m/s.;

outre: *nébulosité* inférieure à 5/10; *évaporation* annuelle autour de 2.000 mm; et *pluie* faible, entre 450 mm et 1.000 mm; en bref, caractéristiques en quelque sorte opposées à celles de la région du *T. infestans*.

Mais il convient de noter que les valeurs citées, de température et humidité, se retrouvent aussi au Sud, où, néanmoins, n'apparaissent pas les deux espèces de «barbeiros» en question. Seul, le vent fort ne se retrouve pas! Et aux latitudes 15°-16°, où domine un vent fort (fig. 12), les deux espèces citées réapparaissent également, bien que la température y soit déjà plus basse.

Récapitulant et résumant, on peut dire que, considérées isolément, les espèces de «barbeiros» existant dans la Vallée du São Francisco se révèlent sensibles, chacune de façon différente, à des valeurs déterminées des éléments climatiques — température, humidité et vent — et que, en lignes générales, parmi les 5 espèces considérées:

Deux sont quasi-cosmopolites: le *P. megistus* et le *T. sordida;*
Une, le *T. infestans,* se localise en région tempérée/humide/calme (Haut São Francisco);
Les deux dernières — *T. brasiliensis* et *T. maculata* — préfèrent une région chaude/sèche/ventée (Moyen-Bas São Francisco).

Il reste à essayer d'élucider l'énigme particulière de l'étroite bande océanique, qui se montre réfractaire à la totalité des 5 espèces de triatomidées, même les cosmopolites.

L'explication suggérée par quelques auteurs, dans le sens d'attribuer cette absence de «barbeiros» à l'humidité des vents maritimes, qui soufflent là constamment, ne s'impose pas, étant donné que dans le Haut São Francisco, où l'humidité est élevée, les triatomidées prolifèrent, et, qui plus est, dans le propre Bas São Francisco, aussitôt

à l'amont de la bande littorale, prévalent les mêmes conditions climatiques que dans cette dernière — vent fort et humidité et température élevées —, ce qui, cependant, n'empêche pas les espèces *megistus, brasiliensis* et *maculata* de s'y multiplier.

Tant qu'à admettre quelque influence, il semble qu'elle doive être cherchée, non seulement dans les caractéristiques proprement météorologiques des vents maritimes régnant là, mais aussi dans la constitution spécifique de *l'air marin* (sel, iode, etc.) que la brise régulière, intensifiée par les alizés dominants, apporte à l'intérieur de la Vallée à une profondeur de 30 km à 40 km.

*Observation finale.* — Il convient d'insister sur le fait que les considérations développées dans ce travail ont un caractère préliminaire, demandant une série de compléments et vérifications, y compris à la lumière de l'examen des valeurs extrêmes et saisonnières des éléments climatiques, à côté des valeurs moyennes, sans parler de la nécessité de faire le test du caractère effectif de l'influence bioclimatologique de la brise de mer dans la bande terminale de la Vallée du São Francisco.

# ZOOGEOGRAFIA DOS TRANSMISSORES DA DOENÇA DE CHAGAS

HERMAN LENT

RESUMO

O A. apresenta, em diapositivos, um estudo sobre a distribuição geográfica das espécies de triatomíneos, os transmissores da doença de Chagas.

Para um total de 88 espécies válidas, segundo o consenso actual do A., são considerados 15 géneros diferentes, dos quais o *Triatoma* Laporte, 1833, contém 50 espécies distintas e os géneros *Rhodnius* Stal, 1859, e *Panstrongylus* Berg, 1879, 10 espécies cada um. As demais espécies estão distribuídas pelos 12 géneros restantes, sempre em número muito reduzido em cada um.

Tendo em vista as regiões zoogeográficas, as espécies são distribuídas:

1 para a Região Paleárctica
3 para a Região Etiópica
5 para a Região Oriental
4 para a Região Australiana
18 para a Região Neo-árctica
64 para a Região Neotrópica

enquanto uma única espécie se distribui por todas às regiões mencionadas.

Os diapositivos que serão apresentados focalizam:

1) A espécie cosmopolita e a distribuição dos vários triatomíneos nas Regiões Paleárctica, Etiópica, Oriental e Australiana, num total de 9 espécies sòmente;
2) As espécies distribuídas nos Estados Unidos da América, por estados;
3) As espécies distribuídas no México, por estados;
4) As espécies distribuídas na Argentina, por províncias;
5) As espécies distribuídas no Brasil, por estados;
6) As espécies distribuídas nos vários países da América Central e nas Antilhas;
7) As espécies distribuídas nos vários países da América do Sul;
8) A distribuição global do género *Rhodnius* Stal, 1859;
9) A distribuição global do género *Panstrongylus* Berg, 1879;
10) A distribuição global do género *Eratyrus* Stal, 1859;
11) A distribuição global dos géneros *Belminus* Stal, 1859, *Bolbodera* Valdés, 1910, *Cavernicola* Barber, 1937, *Parabelminus* Lent, 1943, e *Microtriatoma* Prosen & Martínez, 1952;
12) A distribuição global do género *Psammolestes* Bergroth, 1911;
13) A distribuição global do género *Neotriatoma* Pinto, 1931;
14) Um estudo sobre a distribuição de *Triatoma dimidiata* (Latreille, 1811) e *T. rubrofasciata* (De Geer, 1773) nas Américas;
15) Um estudo sobre a distribuição de 7 espécies diferentes do género *Triatoma* na América do Sul.

# INFORME SOBRE EL PROBLEMA DE LA ENFERMEDAD DE CHAGAS EN EL PERÚ, 1917-1957

HUGO PESCE [1]
y
HUGO LUMBRERAS [2]

Siendo una preocupación de la Oficina Sanitaria Panamericana el problema de la enfermedad de Chagas en las Américas, tal como se señaló en las reuniones de Tucumán, Jujuy y Salta (1949) y en La Habana (1952), fué nuestro deseo contribuir a su conocimiento proporcionando todas las informaciones ocurridas en el Perú desde el año 1917 hasta fines de 1957.

Como se trata de brindar una comunicación sucinta de todos los datos objetivos y numéricos, omitimos, no involuntariamente, una reseña histórica de la enfermedad de Chagas en el Perú y el nombre de los autores que han contribuido a su estudio.

Debemos señalar, sin embargo, que todas las informaciones relacionadas con la enfermedad de Chagas en el Perú no son el resultado de encuestas sistemáticas realizadas en todo el territorio, sino de trabajos aislados de investigadores particulares y ejecutados, sucesivamente, en regiones donde se fué demostrando la presencia de vectores.

Este sucinto informe abarca el estudio de los vectores, del agente etiológico, de los reservorios animales, de los casos humanos, así como algunas consideraciones de caracter epidemiológico.

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## VECTORES

1) Los vectores que juegan papel importante en la transmisión de la enfermedad de Chagas en el Perú son el *Triatoma infestans* y el *Panstrongylus herreri.*

*a*) El *Triatoma infestans* se encuentra distribuido en los valles costeños e interandinos de la región sud y centro-occidental, comprendiendo los Departamentos de Tacna, Moquegua, Arequipa, Ica y Lima. Los índices de infección para el *Schizotrypanum cruzi* son los siguientes: de 1.242 ejemplares procedentes de Tacna el 30,1 por ciento; de 1.169 de Moquegua el 46,8 por ciento; de 2.496 de Arequipa el 36,8 por ciento; de 474 de Ica el 0 por ciento y de 68 de Lima el 0 por ciento. Es decir que de un total de 5.450 ejemplares examinados el promedio de infección global fué de 33,4 por ciento.

*b*) El *Panstrongylus herreri* se encuentra distribuido en el norte del Perú, comprendiendo los Departamentos de Cajamarca, Amazonas y San Martín. Los índices de infección para el *Schizotrypanum cruzi* son los siguientes: de 39 ejemplares procedentes de Cajamarca el 35,8 por ciento; de 73 ejemplares de Amazonas el 45,2 por ciento y de 734 de San Martín el 23,7 por ciento. Es decir que de un total de 846 ejemplares examinados el promedio de infección global fué de 26,1 por ciento.

2) Otros reduvídeos cuya presencia se ha verificado en el Peru son los siguientes: *Triatoma carrioni* en Piura, Amazonas y Cajamarca; *Triatoma dimidiata* en Tumbes y Piura; *Panstrongylus chinai* en Amazonas, Piura, La Libertad y Ancash; *Panstrongylus geniculatus* en Junín y Cajamarca; *Panstrongylus rufotuberculatus* en Tumbes; *Rhodnius pictipes* en Loreto y Cuzco; *Rhodnius robustus* en Loreto; *Rhodnius arcei* n. sp. en Tumbes; *Belminus peruvianus* en Cajamarca y Amazonas y *Eratyrus mucronatus* en Madre de Dios.

3) Se verificó, por primera vez, la infección natural con *Schizotrypanum cruzi* de un ejemplar de *Panstrongylus chinai* entre 15 examinados. Así mismo se comprobó la infección natural del *Triatoma carrioni* por un tipo de tripanosomas que parecen corresponder al *Schizotrypanum cruzi* (un ejemplar entre 15 examinados).

4) Por lo escaso de la recolección de los otros reduvídeos señalados para el Perú no se ha podido investigar si están o no infectados por tripanosomas.

## AGENTE ETIOLÓGICO

1) Se ha comprobado que hay un diferente comportamiento en la virulencia de las cepas de tripanosomas contenidas en el intestino del *Triatoma infestans* de la región sur y del *Panstrongylus herreri* de la región norte del Perú. Pues al hacer inoculaciones en ratas blancas de menos de 30 días de nacidas, con el contenido intestinal de tales reduvídeos naturalmente infectados, se observó que la cepa del sur produce una parasitemia larga e intensa, mientras que la cepa del norte selvático la produce corta y escasa.

2) La cepa de *Schizotrypanum cruzi* obtenida del *Panstrongylus chinai,* naturalmente infectado, al ser inoculada en ratas blancas tiene un comportamiento muy semejante a la obtenida del *Triatoma infestans* del sur. En cambio la cepa de trypanosomas observada en el contenido intestinal del *Triatoma carrioni,* naturalmente infectado, cuando se le inoculó en tres ratas blancas tiernas no dió resultado positivo hasta los 36 días que duró el control.

## RESERVORIOS ANIMALES

1) Se han señalado como reservorios animales del *Schizotrypanum cruzi* en el Perú a los cobayos, perros y gatos. Así: de 779 cobayos, 88 perros y 31 gatos examinados por el método de examen directo de sangre se comprobó que el 5,9 por ciento, el 3,4 por ciento y el 3,2 por ciento, respectivamente, estaban infectados.

2) El examen de otros animales domésticos, como conejos, ratones de campo y cerdos, dieron resultados negativos para el *Schizotrypanum cruzi.*

3) Los estudios anotados sobre reservorios animales se refieren a trabajos realizados en la región sud-occidental del Perú. Se sospecha que en la región selvática del norte éstos y otros animales más jueguen papel de reservorios del agente de la enfermedad de Chagas.

## CASOS HUMANOS

1) Hasta fines de 1957 se han verificado en el Perú un total de 122 casos de enfermedad de Chagas en el hombre, distribuidos en la siguiente forma: 42 en Tacna; 15 en Moquegua; 35 en Arequipa; 22 en Amazonas; 2 en San Martín; 3 en Cajamarca; 1 en Madre de Dios; 1 en Junín y 1 en una región del norte no determinada.

2) De todos los casos de enfermedad de Chagas 113 fueron confirmados por xenodiagnóstico, 7 por examen directo de sangre, 1 por cultivo y otro por método clínico epidemiológico.

3) No se han estudiado los índices de morbilidad ni mortalidad de la enfermedad de Chagas en las zonas rurales endémicas. Solamente se puede señalar que de 1.141 xenodiagnósticos aplicados indiscriminadamente entre gente que vive en las regiones endémicas el 6,4 por ciento dió resultado positivo y de 250 aplicados entre sujetos que habitan viviendas de condiciones epidemiológicas favorables para la existencia de la enfermedad de Chagas el 14,8 por ciento resultaron positivos.

4) No se han hecho estudios sobre la evolución clínica de los pacientes con enfermedad de Chagas confirmada y recién, en los últimos tiempos, se ha comenzado a investigar las alteraciones cardíacas que pueden ocurrir. Por otra parte no se han registrado casos de evolución fatal debido muy probablemente a las dificultades para el diagnóstico en las regiones rurales y a la falta de comprobaciones histopatológicas.

## CONSIDERACIONES EPIDEMIOLÓGICAS

1) Las condiciones epidemiológicas en las regiones rurales endémicas del Perú son propicias para el desarrollo y propagación de la enfermedad de Chagas.

2) El tipo de construcción de la vivienda rural es de condiciones paupérrimas; pues en general las viviendas son reducidas, poco ventiladas e iluminadas, construidas de material de piedra, sillar, carrizo, madera, techos de paja, caña u hojas de árbol y, por lo tanto, llenas de huecos y resquebrajaduras, donde se refugian los reduvídeos después de alimentarse en el hombre o en los animales domésticos que,

en un alto porcentaje, se crían en la misma vivienda o muy cerca de ella.

3) Se ha comprobado que un 42 por ciento de un total de 1.145 viviendas de los valles de Tacna, Moquegua y Arequipa se encuentran infestadas por el *Triatoma infestans* y que un 52,1 por ciento de las viviendas de la región selvática del norte se encuentran infestadas por el *Panstrongylus herreri.*

4) En relación a los reservorios se puede señalar que en más del 88 y 85 por ciento de las viviendas de la costa y de la selva, respectivamente, se crían numerosas y variadas especies de animales domésticos. Así, los porcentajes parciales de las viviendas que crían diferentes tipos de animales son los siguientes:

*a*) En la costa del sur, comprendiendo Arequipa y Tacna, en el 50,6 por ciento crían cobaios, en el 59,7 por ciento perros, en el 41,8 por ciento gatos y en el 73,3 por ciento aves, sobre un total de 454 viviendas rurales registradas para este fin.

*b*) En la región selvática del norte, comprendiendo solamente San Martín, en el 71,1 por ciento se crían cobayos, en el 34,8 por ciento perros, en el 2,1 por ciento gatos y en el 80,7 por ciento aves, sobre un total de 329 viviendas rurales registradas con este fin.

5) El descuido de la educación sanitaria es culpable en mucho de que los pobladores de las zonas rurales tengan un nivel de vida higiénico-sanitario bastante paupérrimo y por lo tanto favorable para la propagación de las endemias.

## CONSIDERACIONES GENERALES

1) La enfermedad de Chagas es una afección rural y endémica de los valles costeños e interandinos del sur del Perú, así como de la región selvática del norte.

2) Los insectos vectores, por sus características biológicas, su fácil vehiculización a otras regiones, su adaptabilidad a diversos climas y diversos huéspedes de los cuales se alimenta, se están extendiendo con rapidez a zonas donde no se les conocía y amenazan, por lo tanto, difundir más la enfermedad de Chagas, creando un grave problema de salud pública.

3) Como el estado de la enfermedad de Chagas en casi todos los países de América donde existe es muy semejante al del Perú, conviene, por tratarse de una endemia peligrosa, que los miembros de este IV Congreso recomienden a los organismos internacionales de salud de que no solamente pongan su atención a este problema, sino que, a la brevedad posible, presten toda su ayuda técnica y económica para lograr su mejor conocimiento y, sobre esa base, estructurar los planes de una futura pero pronta erradicación.

# A COMPARISON OF STRAINS OF *TRYPANOSOMA CRUZI* INDIGENOUS TO THE UNITED STATES WITH CERTAIN STRAINS FROM SOUTH AMERICA

FRANS C. GOBLE

It does not seem necessary for me to spend any time in review of the many things we all know about the history, etiology and epidemiology of Chagas' disease, because this audience is well informed and the preceding papers have been so informative. I should like, therefore, to consider here only some of the more recent findings concerning the distribution of *Trypanosoma cruzi* in the United States and to report on our studies made in an attempt to characterize some of the recently isolated strains.

Up until 1955 the presence of Chagas' disease north of the Mexican border had been established only by a few serological records and electrocardiographic studies. The causative agent had been found in triatomes as early as 1916 in California. The most northern record of the parasite in bugs was from that state and the most eastern record in bugs was from Texas, where the serological cases occurred.

In 1955 the first autochthonous case of human trypanosomiasis in the United States, in which parasites could be demonstrated and cultured, was reported by Woody (Fig. 1). This occurred in a child in Corpus Christi, Texas, and it was noted that both bugs and armadillos were common in the vicinity. A second indigenous case, also diagnosed in a child in 1955, was from Bryan, in Brazos County, Texas, which is about 200 miles farther north.

In 1956, raccoons *(Procyon lotor)* trapped at Patuxent, Maryland, were found to harbor *Trypanosoma cruzi* (Walton *et al.*). This area is the most northerly and most easterly habitat reported for the organism in North America. In 1957, related trypanosomes were isolated from

raccoon, skunk, and opossum in the southern part of Georgia (Brooke, *et al.*), and subsequently the gray fox was also found to be a host (McKeever, Gorman & Norman). In this last study almost 2,000 animals were examined during a survey for leptospirosis, both blood and kidney cultures being taken. Trypanosomes were isolated from about 5 percent of the animals, the highest incidence occurring in oppossums. It was found that 16 percent of the kidney cultures from oppossums were positive, but only 6 percent of the blood cultures.

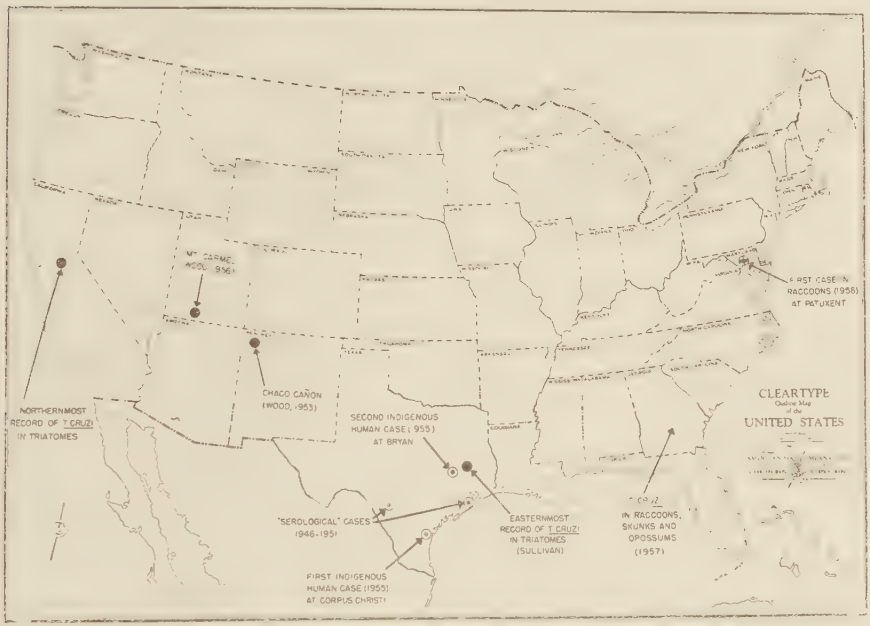


Fig. 1 — Recent records of *T. cruzi* in mammals in the United States in relation to previous records in invertebrate vectors

The parasites were grown from about 7,5 percent of the urine samples tested. The occurrence of the organism in the urine is of great interest in suggesting the possibility of another mode of transmission in areas (such as these in the eastern United States) where the insect vectors are relatively scarce.

The raccoon records add the *Procyonidae* to the list of carnivorous families known to harbour *T. cruzi* and the skunk *(Mephitis mephitis)* is a new representative of the *Mustelidae* to be reported as a host.

Our continued interest in strain differences led us to undertake a study of these recently isolated strains in an attempt to characterize them in terms of infectivity and pathogenicity. In view of certain experiences of our own and of others, it was felt that information obtained in mouse infections could not easily be applied to the prediction of pathogenicity for other animals, and that we should therefore use some other animal which might present a more natural host-parasite relation. Fatal experimental infections can be produced in certain strains of mice and in some other animals which are not normal hosts of *T. cruzi,* but most animals do not suffer from natural infections. Dogs and cats, however, do sometimes become ill with the disease and dogs sometimes die as a result of the infection (Dias, 1954). The dog has, therefore, been considered to be a particularly interesting host both epidemiologically and pathologically (Goble, 1954).

We studied, therefore, in this species, the infectivity and pathogenicity of the strains from the first two indigenous human cases of Chagas' disease in the United States, and from the first raccoon cases, in comparison with two strains from South America, one from Brazil, the other from Chile. The strains and their origins are shown in the table. All of the strains were maintained in culture and all

| Strains | Origin | Date | Host |
|---|---|---|---|
| «Brasil» . . . . . | Brasil | 1942 | *Homo sapiens* |
| «Tulahuen» . . . . . | Chile | 1945 | *Triatoma infestans* |
| «Corpus Christi» . . . . . | Texas | 1955 | *Homo sapiens* |
| «Houston» . . . . . | Texas | 1955 | *Homo sapiens* |
| «Patuxent» . . . . . | Maryland | 1956 | *Procyon lotor* |

of the dogs, which were born and raised in the laboratory, were infected at exactly 10 weeks of age by subcutaneous injection of culture organisms. A number of litters were used in order to minimize the possibility of genetic variations in susceptibility.

Time will not allow for a detailed presentation of the findings during these studies. As observations accumulated, we felt the need

for a method of expressing the information we had on infectivity and pathogenicity in some graphic way which would allow for rapid visualization of the strain differences without reference to the *data minuta*. As a result a scheme was developed (Fig. 2) in order to show at a glance the characteristic course of the disease for each

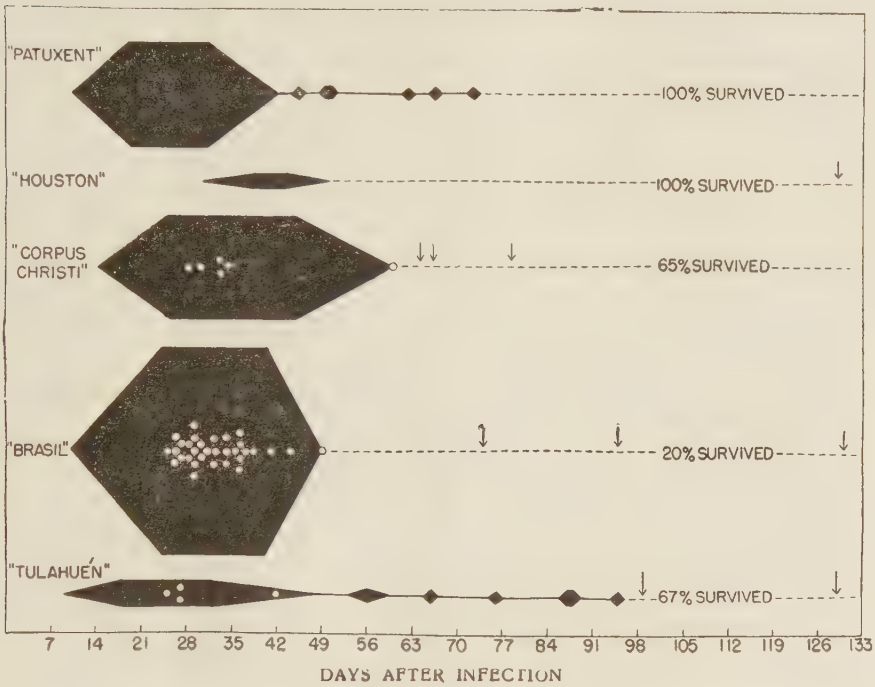


Fig. 2 — Patterns of patency of experimental Chagas' disease in dogs infected with various strains of *T. cruzi*. The dark areas represent the patent periods, their vertical dimension indicating the relative parasitemias attained. The white circles represent the acute cardiac deaths and the arrows indicate points at which animals were sacrificed during the post-patent period

of the 5 strains. The earliest and latest days of patency, the days of the earliest and latest parasitemic peaks, the relative degrees of parasitemia attained, and the days on which deaths occurred (white circles) are illustrated.

The relative degrees of parasitemia between the strains are not shown to scale because the differences were too great. The highest

parasitemia observed in infections with «Brasil» was 750 times that observed in infections with «Houston», but the figure indicates only a 16 fold difference.

The feeling, expressed by von Brand *et al.* (1949), that tissue invasiveness (and thence pathogenicity) is correlated with the number of parasites appearing in the peripheral blood was corroborated in only a general way by our observations. «Brasil», which produced the highest parasitemias, produced the most mortality, and «Houston», which produced the lowest parasitemias, caused no deaths. When the strains which effected intermediate parasitemias are compared, however, the correlation is not apparent. Roughly similar parasitemic levels were attained by both «Corpus Christi» and «Patuxent», and these levels were several times greater than those attained by «Tulahuen», but «Patuxent» caused no deaths and the mortality resulting from «Corpus Christi» infections was no greater than that produced by «Tulahuen».

The cause of death in all of the dogs which succumbed was acute myocarditis and heart failure. In this respect the animals infected with «Tulahuen» did not differ from those infected with «Brasil» or «Corpus Christi». Taliaferro and Pizzi (1954) have already pointed out that the designation of the «Tulahuen» strain as reticulotropic, while appropriate, is not completely descriptive because the parasite certainly invades many non-reticular tissues. Animals infected with «Houston» and «Patuxent» were sacrificed in the prepatent and patent periods in order to obtain tissues which might contain parasites. No intracellular forms of «Houston» were found. Foci of leishmanioid stages of «Patuxent» were found in the heart muscle, but could not be discovered in any other organ examined.

No deaths occurred in the post-patent period and no relapse occurred later than the 95th day with any of the strains. Some of the survivors were sacrificed at various times (indicated by the arrows on the chart), during the post-patent period, and tissues obtained at necropsy were examined for parasites and lesions. Neither were found in any of the animals. No tissue changes suggestive of chronic parasitic heart disease were seen, but electrocardiographic studies, which might have revealed functional disturbances, such as Laranja *et al.* (1954) have reported in chronic canine Chagas' disease, were not done.

It should be pointed out that all of the infections reported here were induced by inoculation of culture organisms, which represent the morphological and physiological stages comparable to those which occur in the invertebrate vector. We feel this should be emphasized as representing a host-parasite relationship somewhat different from the infections studied by Taliaferro and Pizzi (1955) in which blood parasites adapted to a particular highly inbred strain of mice are used as inoculum. Although the use of mouse adapted strains of *T. cruzi* in genetically homogenecus mice has made possible many interesting studies during the past few years, it is as yet difficult to say with certainty how much of the information obtained in such systems can be applied to the probiems of virulence in man or other animals.

Although we have felt that the use of culture forms as inoculum results in infections which are more likely to resemble those which might be expected in nature, there are of course many things we do not know about the organisms as they are maintained outside the definitive host. It is not known how closely maintenance in culture may simulate maintenance in the intermediate host or what physiological changes accompany loss of virulence. Von Brand *et al.* (1949) studied the cultural behavior of 4 strains of *T. cruzi* of different degrees of virulence for rats, and reported that no distinct differences could be observed in their physiology. We cannot elaborate on this interesting subject, but we should like to summarize now and to express some opinions as to the potential infectivity and pathogenicity of the strains of *T. cruzi* we have investigated.

It is our feeling that the «Houston» strain, which has very low infectivity for mice, and is completely avirulent for dogs, is probably not one which would show any signs of its presence in a healthy individual. The patient from whom it was isolated was an infant who had apparently suffered from a *Salmonella panama* infection from the age of only two and one-half weeks. In spite of all therapeutic efforts, this bacterial infection invaded the spinal fluid, which remained positive for several weeks. Obstructive hydrocephalus was a complication, and it was during post-operative laboratory examination of fluid from the left ventricle of the brain that the trypanosomes were first noted. It seems quite possible that *T. cruzi* was, in this

case, only a coincidental inhabitant of the child and not etiologically connected with his illness.

On the other hand, the «Corpus Christi» strain has shown definite pathogenic proclivities in experimental animals, causing in dogs a mortality about the same as that produced with the «Tulahuen» strain. It must, however, again be pointed out that it is possible that it was not the causative agent of the clinical conditions noted in the patient. The salutory effect, observed in this child (Woody and Woody, 1955), following the administration of tetracycline, which is not active against *T. cruzi*, would seem to indicate the possibility of a concurrent bacterial infection being responsible for the signs and symptoms reported (Goble, 1956). Lesser and Lukeman (1957) have studied this strain in mice and, although they have not published completely, they noted in their animals the presence of necrosis in the upper third of the stomach, and, on microscopic examination, they found leishmanioid forms of *T. cruzi* in the serosa and muscle layers. We have not observed any gross lesions in the stomachs of dogs and the significance of the presence of the intracellular parasite in the walls of the alimentary tract, which is considered by some (Köberle, 1958; Porto, 1955) to be of great pathological importance, has yet to be fully elucidated.

Recently Moore (1957) has made a preliminary study of the antigenic composition of the culture forms of the «Corpus Christi» and «Houston» strains in comparison with «Brasil» by means of the Ouchterlony gel diffusion technique. By these methods it appeared that the «Houston» strain shared with the «Brasil» strain one antigenic component which the «Corpus Christi» strain did not possess, but there were in the «Brasil» strain several components not detectible in the North American strains, including some which gave cross reactions with *L. tropica*. A correlation between antigenic composition and infectivity or pathogenicity is, of course, yet to be established.

The «Patuxent» strain, although capable of producing parasitemias as high as those caused by the «Corpus Christi» strain, has not killed any of the animals in our experiments. Diamond & Rubin (1956) have studied this strain in day-old pigs, lambs, and goats and found it infective for all 3 species. In sheep and goats a low grade short term parasitemia ensued. In pigs parasites were not observed in the blood. Latent infections were detected by blood cultures and they

persisted for at least 8 weeks in pigs and sheep, and 4 weeks in the goats. None of these species showed any sign of the disease and no gross lesions were observed at autopsy, although leishmanioid forms were found in the heart muscle of the pig and goat. Unfortunately so little is known about the effects of any other strain of *T. cruzi* in artiodactyls that it is impossible to say whether the low pathogenicity is attributable to the characteristics of the strain of parasite or of the host. Walton *et al.* (1956) produced experimental infections with the «Patuxent» strain in raccoons, mice, and rats by means of inocula from blood, culture, and *Triatoma protracta*. Infected raccoons developed high complement-fixation titers, reacting with antigens produced from «Brasil» strain.

It is obvious that we have been able to consider here only a few of the many interesting recent researches on *T. cruzi*. Studies which have continued unremittingly in Latin America are being reported in another section of these Congresses. Although it sometimes seems that what we *do not* know about Chagas' disease after fifty years of study is almost as impressive as what we *do* know, each year our accummulating knowledge brings us closer to a complete understanding of this intricate and intriguing zoonosis.

## BIBLIOGRAPHY


1. Brooke, M. M., Norman, L., Allain, A., and Gorman, G. W. — «Isolations of *Trypanosoma cruzi*-like organisms from wild animals collected in Georgia», *J. Parasitol.*, **43** (5, sect. 2): 15, 1957.
2. Diamond, L. S., and Rubin, R. — «Experimental infections of certain farm mammals with a North American strain of *Trypanosoma cruzi* from the raccoon». *J. Parasitol.*, **42** (4, sect. 2): 21, 1956.
3. Dias, E. — «Chagas-Krankheit. Chagas' Disease», *Weltseuchenatlas*, edited by E. Rodenwaldt, Heidelberg, published by Falk-Verlag, Hamburg. Part II: 135-140, 1954.
4. Goble, F. C. — «Thyroid changes in acute experimental Chagas' disease in dogs», *Am. J. of Pathology*, **30** (3): 599-607, 1954.
5. Köberle, F. — «Megacolon», *J. Trop. Med. and Hyg.*, **61** (1): 21-24, 1958.
6. Laranja, F. S., Dias, E. Nóbrega, G., and Miranda, A. — «Chagas' disease: a clinical, epidemiologic, and pathologic study», *Circulation*, **14** (6): 1035-60, 1956.
7. Lesser, E., and Lukeman, J. M. — «Stomach infections with *Trypanosoma cruzi*», *J. Parasitol.* **43** (1): 65, 1957.

8. McKeever, S., Gorman, J. W., and Norman, L. — *Occurrence of a* Trypanosoma cruzi*-like organism in some mammals from Southwestern Georgia and Northeaster Florida* (in press), 1958.
9. Moore, D. V. — «Antigenic relationships of certain hemoflagellates as determined by the Ouchterlony gel diffusion technique», *J. Parasitol.*, **43** (5, sect. 2): 16, 1957.
10. Porto, C. — «Gastropatia chagásica crónica — Nota prévia», *Rev. Goiana de Med.* **1** (1): 43-54, 1955.
11. Seneca, H., and Wolf, A. — «*Trypanosoma cruzi* infection in the Indian monkey», *Amer. J. Trop. Med. and Hyg.*, **4** (6): 1009-14, 1955
12. Taliaferro, W. H., and Pizzi, T. — «Connective tissue reactions in normal and immunized mice to a reticulotropic strain of *Trypanosoma cruzi*», *J. Inf. Dis.*, **96** (3): 199-226, 1955.
13. Walton, B. C., Bauman, P. M., Diamond, L. S., and Herman, C. M. — «*Trypanosoma cruzi* in raccoons from Maryland», *J. Parasitol.*, **42** (4, setc. 2): 20, 1956.
14. Woody, N. C., and Woody, H. B. — «American trypanosomiasis (Chagas' disease). First indigenous case in the United States», *J. Amer. Med. Assoc.*, **159** (7): 676-7, 1955.

# CHEMOTHERAPY OF CHAGAS' DISEASE

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From the beginning of chemotherapy, investigators have looked for substances that could be effective against infections produced by trypanosomes.

The importance given since then to the research of trypanosomacidal substances is justified by the frequent occurrence of this disease in the African continent, hindering the work of its population in the development of its natural resources. The discovery of the American trypanosomiasis produced by *Schizotrypanum cruzi,* which is widely disseminated and attacks seriously the rural population of a great part of the American continent, contributed to a greater interest in this research.

There are fundamental differences between the biology of the African pathogenic trypanosomes and the biology of *S. cruzi,* causal organism of Chagas' disease; these differences do not allow for the use of the same methods of chemotherapeutical investigation. *T. gambiense, T. brucei, T. congolense* and *T. vivax* show a *continuous* multiplication in the vertebrate host and the division occurs during the trypanosomal phase in the blood and lymph circulation. In *S. cruzi,* the multiplication in the mammals is *discontinuous,* mainly in the leishmanial phase in the tissue cells.

These facts advise against the verification of the chemotherapeutical effect of substances as regards the infection produced by *S. cruzi,*

by basing it on the number of parasites found in the blood, being this the criterion adopted by some authors.

We have observed that the experimental infections by *S. cruzi* in laboratory animals, with the strains we have, tends to chronicity or perhaps to a spontaneous cure if the animals can survive the visceral lesions. We have also noticed that the infection produced by this trypanosome aggravates the latent infections by virus or by bacteria, which are very common in mice, causing a high mortality. If we eliminate that frequent cause of error, the survival or the infected animals would be very great.

The investigation of the bacteriostatic activity of certain substances *in vitro,* using bacterial cultures, has supplied valuable information to chemotherapy; however, this has not occurred in the study of substances that may have a trypanosomacidal activity. This is due to the fact that trypanosomes, especially *S. cruzi,* are organisms of a cellular constitution more differentiated than that of bacteria; consequently, they are more sensitive to variations in the culture media and these influences, either do not exist in the infected organism or trypanosomes are not influenced by them in a more favorable medium to their development.

Magarinos Torres suggested that we should adopt, in our chemotherapeutical investigations of *S. cruzi,* the criterion of using the characteristic myocarditis produced by Chagas' disease as a reference for evaluating the activity of the tried substances. This criterion, being an objective method, has given us valuable information by the verification of typical lesions that may be compared among themselves as against the statistical methods, based on the parasitemia and on the animals' survival.

Chemotherapy aims increasingly at finding substances that can act directly, hindering the metabolism of the invading cell of the infested body. With this scope we must find a metabolism that can be ascribed to *S. cruzi,* and which can be influenced by the use of antimetabolites.

Several authors refer to the fact of *S. cruzi* selecting the cardiac fiber for its multiplications as due to a tropism provoked by substances existing in the myocardium. *S. cruzi* has allergenic properties (1) and this statement can be immediately justified if we consider the capacity of this trypanosome to induce the development of antibodies observed

in different immunogenic reactions that have been related. Such facts suggest (2) that the allergic reaction provoked by the presence of trypanosomes in the body, from which myocarditis would result (3), would favor their development because the liberated circulating histamine and the histamine bound to the tissue protein constitute a factor favorable to their multiplication.

The penetration of *S. cruzi* in the body acting as an allergen would provoke a histamine discharge in the blood, contributing to the permanence of the trypanosomes in the circulation, until the appearance of allergic reactions in certain organs would provoke the visceral localization of that trypanosome.

We have had an opportunity to relate (2) a cycle where *S. cruzi* would utilize in its development metabolites derived from histamine. To obtain these metabolites it would use 3 enzimes that it would possess:

1 — By means of *protease* (proteolytic enzyme) it would produce the hydrolysis of the organic tissue proteins, liberating the amino acid histidine;
2 — By means of the *histidine-decarboxylase* enzyme it would transform the amino acid histidine into histamine;
3 — By means of the *diamine oxidase* enzyme (histaminase) it would convert histamine into metabolites which would favor its growth.

In support of this hypothesis, we can mention the following facts:

*a*) The allergenic nature of *S. cruzi* is very marked in some strains of this trypanosome. This allergenic nature is responsible for the appearance of the interstitial myocarditis, the intensity of which does not correspond to the presence of trypanosomes in the tissues nor to the presence of these parasites in the blood (3);
*b*) The production of myocarditis by the development of an allergic reaction after sensitization by the injection of dead *S. cruzi* (1);
*c*) The favorable action of *S. cruzi* on the growth of *Endamoeba histolytica* in culture media, to which a great number of dead

trypanosomes is added when it is known that only this trypanosome (excluding other trypanosomes) and *Escherichia coli* are able to supply the elements that favor the large multiplication of *E. histolytica* in the culture media (4); it is also known that *E. coli* has proteolytic enzymes able to liberate histidine from the tissues and another enzyme, the decarboxylase histidine, able to transform histidine into histamine, as well as the histaminase enzyme (5); thus, it would be reasonable to suppose, in view of its behavior in relation to *E. histolytica,* that *S. cruzi* also has those same enzymes;

*d*) The prevailing presence of histidine and histamine in the intestinal contents of triatomes (6, 7), thus explaining why the proliferation of *S. cruzi* is so intense in the intestines of those vectors of Chagas' disease.

In addition, we can mention here that there is evidence that the African trypanosomes have, in a much more restricted degree, the capacity of utilizing the metabolites derived from histamine in their development.

Physiologists call histamine liberators the substances that, when introduced into the body, cause the liberation of the histamine stored in the tissues which, liberated in this manner from its combination with the cellular protein, passes into the circulation.

It is worthy of notice that the more active trypanosomacides presently in use are active histamine liberators. Histamine liberators are: stilbamidine, propamidine and pentamidine (8); the first is active against blastomycosis, leishmaniasis and Indian kala-azar, but less active than the other two against the African trypanosomes and also less active as a histamine liberator; the second is active against *Trypanosoma rhodesiense* and *T. gambiense;* the third is active against these trypanosomes and also against *T. equiperdum.*

These substances would act as histamine liberators, depriving the tissues (and probably the trypanosome itself) from the necessary histamine for its growth. They would not act, however, inhibiting the growth of *S. cruzi,* because this trypanosome, contrary to the others, would have its own enzymes able to provoke the protein hidrolysis up to the last phase of the formation of metabolites derived from histamine.

The hidrazide of isonicotinic acid (isoniazid) is an inhibitor of the histaminase and its action is proportional to the dose used; this hydrazide also inhibits the decarboxylase histidine produced by *E. coli* (9). All the basic antibiotics, and the basic synthetic antituberculotic substances tested up to the present, reduce the activity of the diamine oxidase (histaminase) of bacterial origin (10).

Substances capable of inhibiting the action of the diamine oxidase (histamie) such as the isoniazid and the antibiotics of basic nature — for instance, the tetracycline derivatives; aureomycin (11) and terramycin — prove to be able to favor *S. cruzi* growth, mainly its multiplication in the myocardium (isoniazid) (12). This fact may have the following explanation: there are, at least, two processes utilized by the organism to avoid the excess of free histamine in the circulating blood and organic fluids; in the first of these processes, free histamine is destroyed by diamine oxidase, and the second process consists in the storage of histamine bound to the tissue protein. Certain substances are capable of liberating the histamine stored in the tissues, inducing the action of proteolytic enzymes.

*S. cruzi,* acting as an allergen, provokes a histamine reaction in some organs, especially in the cardiac fiber; with the occurrence of the diamine oxidase blocked by the isoniazid or by the basic antibiotics, aureomycin and terramycin. the trypanosome would meet elements favorable to an intense proliferation, not only in the circulating histamine which ceases to be destroyed by the blocked histaminase, but also in the histamine retained in the tissues by a process of physiologic balance.

In the experiments we have conducted in mice inoculated with *S. cruzi* and treated orally with isoniazid we have noticed a marked increase in myocarditis and the presence of numerous agglomerations of leishmanial corpuscles in the cardiac fiber, in opposition to the inoculated control animals that showed less extensive lesions (12). That remarkable increase in the number of trypanosomes in the heart contrasted with the reduced number of parasites in the circulating blood.

No doubt there are in the triatomes vectors of *S. cruzi* substances capable of promoting the multiplication of this trypanosome within the intestinal tract. In a former work, in collaboration with Barth, Tavares and Pereira (6), by means of paper chromatography, we

had the occasion of noticing the presence of free histidine and free histamine in the intestinal contents of the triatome to which we attribute the favorable condition to the trypanosome multiplication in the triatomes. Harington noticed the existence of remarkable quantities of histamine and histidine in the feces of the triatome *Rhodnius prolixus* (7). Facing the arguments mentioned, we can conclude the existence of a close relation between the *S. cruzi* development and the histamine present in the body. It remains, however, to find out an explanation to the fact of the multiplication forms of these trypanosomes, which are constituted by colonies of leishmanial corpuscles, being localized predominantly in the muscular fibers. An analogy of this occurrence is found in the histaminic metabolism of *Clostridium welchii* and *E. coli* (5). It was ascertained that both *Cl. welchii* and *E. coli,* when cultivated in the presence of glycogen, produce a decarboxylase histidine that leads to the histamine formation. As Gale (13) notices, «it seems probable that, when *Cl. welchii* invades the muscular tissues, conditions appear which are favorable to the histamine production, as the fermentation of the muscle glycogen would produce the necessary low pH, especially in circumscribed areas in such as heterogeneous medium; and the body proteolytic activities would liberate the histidine».

We have verified in our experiments that substances that increase the quantity of stored glycogen provoke the appearance of leishmanial corpuscles in the muscular tissues. Such is the case verified by us in animals injected with hydrocortisone.

Pizzi and col. (15) observed that high doses of cortisone administered for some time aggravate the course of the experimental infection by *S. cruzi.* It is known that the 11-oxysterols (hydrocortisone, cortisone) are more effective diabetogenic agents than the other sterols and they also provoke a greater deposition of glycogen in the liver of the treated animals (16).

The glycogen of the heart tends to be maintained within normal or increased values under conditions that cause a severe decrease in the glicogen of the liver and skeletal muscles. The maintenance of the levels of the glycogen of the heart in adverse circumstances is in harmony with the vital function of this organ — West and Todd (14).

Schern showed the influence that glucose has on the trypanosomes' vitality. Jancso and Jancso, having verified that germanine (Bayer 205) inhibits the metabolism of the trypanosomes' carbohydrates, tested hypoglycemic substances derived from guanidine, and verified that synthline acts inhibiting the trypanosomes' multiplication in infected mice. It was the knowledge of this fact that gave place to investigations from which the introduction of stilbamidine, propamidine and pentamidine in therapeutics resulted; we had already mentioned them as active histamine liberators and, besides being hypoglycemic substances, they also are trypanosomacides.

The trypanosomacidal activity shown by some hypoglycemic substances seems not to be related to the reduction of the glucose blood levels because it was verified that insulin lacks altogether any trypanosomacidal action; consequently, it would be reasonable to suppose that those substances, which intervene in the glucose metabolism in the body cells, would intervene in a similar way in the glucose metabolism of the trypanosomes' cell itself. This would not occur with the insulin, which has a direct action on the glucose which is destroyed by oxidation.

With the aim of proving those facts, it has occurred to us to test the action of a hypoglycemic substance, belonging to another class of chemical substances, but that also has a hypoglycemic action similar to that of guanidine derivatives.

We have thus ascertained that $N_1$-sulfanill-$N_2$-n-butilcarbamide (BZ 55) administered orally to mice has a definite influence on the interstitial myocarditis produced by *S. cruzi,* diminishing clearly its intensity (17).

## SUMMARY

1. The infection by *S. cruzi* produces lesions of an allergic nature in the animal body, as, for example, the interstitial myocarditis, that can he reproduced experimentally by the inoculation of dead trypanosomes.

2. Several experimental facts permit the attribution to *S. cruzi* of a metabolic cicle in which, by means of 3 enzymes (protease, histidine decarboxylase and histaminase), metabolites derived from histidine would be obtained and would be utilized by this trypanosome for its growth.

3. In this cycle, the glycogen deposited in the muscles, especially in the myocardium, plays an important part, favoring the proteins digestion.

4. Substances that inhibit the histaminase action (isoniazid, aureomycin, terramycin) aggravate the infection produced by *S. cruzi*. In the same way, the substances that increase the glycogen deposition in the body (hidrocortisone, cortisone) aggravate that infection.

5. Certain substances possessing a hypoglycemic action (Bayer 205 and the diamidines: synthaline, stilbamidine, propamidine and pentamidine) have a trypanosomacidal action on the African trypanosomes which are more adapted to the parasitic life than *S. cruzi* and, therefore, more sensitive to the antimetabolic action of those substances.

6. We noticed that $N_1$-sulfanilil-$N_2$-n-butilcarbamide (BZ 55), which shows a marked hypoglycemic action, has a definite influence on the diminution of the intensity of the interstitial myocarditis produced by *S. cruzi* on infected mice.

## BIBLIOGRAPHY


1. Muniz, J., e Penna de Azevedo, A. — «Novo conceito da patogenia da doença de Chagas *(Tripanosomiasis americana)*», *O Hospital*, **32**: 165, 1947.
2. Botafogo Gonçalves, N., Silva Carmo, E., e Tavares, B. M. — «Novas bases para a quimioterapia da moléstia de Chagas», *Rev. Bras. Med.*, **13**: 81, 1956.
3. Magarinos Torres, C. — «Patogenia de la miocardite crónica en la enfermedad de Chagas», Quinta reunión de la Soc. Argentina de Pat. Reg. del Norte, 902, 1929.
4. Phillips, B. P. — «Comparative effects of certain species of *Trypanosomidae* on the growth of *Entamoeba histolityca* in the absence of bacteria», *Am. J. Trop. Med.*, **31**: 290, 1951.
5. Gale, E. F. — «The production of amines by bacteria — I. The decarboxylation of amino acids by strains of *Bacterium coli*», *Biochem. J.*, **34**: 392, 1940.
6. Botafogo Gonçalves, N., Barth, R., Tavares, B. M., e Duarte Pereira, E. — «Cromatografia, em papel, do conteúdo intestinal dos triatomas», *Ciência e Cultura*, **8**: 187, 1956 (trabalho apresentado a SBPC, reunião de Ouro Preto, em 5-7-56).
7. Harington, J. S. — «Histamine and histidine in excreta of the bug *Rhodnius prolixus*», *Nature* (London) **178**: 268, 1956.
8. Riley, J. F. — «The effects of histamine-liberators on the mast cells of the rat», *J. Path. Bact.*, LXV: 471.
9. Baur, H., and Staub, H. — «Tuberculostatica (Isonicotin hydrazid u. a.) und Histaminstoffwechsel», *Helvetia Physiologica et Pharmacologica Acta*, **11**: c-9, 1953.
10. Zeller, E. A., Barsky, J., Fonts, J. R., Kirchheimer, W. F., and van Orden, L. S. — «Influence of isonicotinic acid hydrazide (INH) and 1-isonicotynil-2-isopropyl hidrazide (IIH) on bacterial and mammalian enzymes», *Experientia*, **8**: 349, 1952.

11. JARPA, A., CHRISTEN, R., AGOSIN, M., y PIZZI, T. — «Ensayos de quimioterapia en la enfermedad de Chagas experimental. I — Aureomicina», *Bol. Inf. Parasit. Chilenas,* **4**: 49, 1949.
12. BOTAFOGO GONÇALVES, N., TAVARES, B. M., e SILVA CARMO, E. — «Acção da isoniazida na infecção experimental pelo *Schizotrypanum cruzi*», *Anais da Acad. Brasil. de Ciências,* **28**: 229, 1956.
13. GALE, E. F. — «The decarboxylation of amino acids by organisms of the groups *Clostridium* and *Proteus*», *Biochem. J.,* **35**: 77, 1940.
14. WEST, E. S., and TODD, W. R. — *Biochemistry,* p. 964, Macmillan Co., 1951.
15. THIERMANN, E., y CHRISTEN, R. — «Influencia del acetato de cortisona y de la aureomicina como agente agravador de la infección chagásica experimental», *Bol. Inf. Parasit. Chilenas,* **7**: 53, 1952.
16. PIZZI, T., RUBIO, M., PRAGER, R., y SILVA, R. — «Acción de la cortisona en la infección experimental por *Tripanosoma cruzi*», *Bol. Inf. Parasit. Chilenas,* **7**: 22, 1952.
17. RUSSEL, J. A., and WILHELMI, A. E. — *Physiology of the adrenal cortex in medical uses of cortisone,* edited by F. D. W. Lukens, Blakiston Co., 1954.
18. MAGARINOS TORRES, C., BOTAFOGO GONÇALVES, N., TAVARES, B. M., e GONÇALVES, F. — «Acção da $N_1$-sulfanilil-$N_2$-n-butilcarbamida (BZ 55) sobre a miocardite intersticial produzida pelo *Schizotrypanum cruzi*», *Anais da Acad. Bras. de Ciências,* Julho de 1957 (in press).

## GENERAL DISCUSSION

Doctor Luis Espinoza (Ecuador) — Deseo felicitar al Prof. Emmanuel Dias por su valiosa y brillante comunicación sobre el problema de la enfermedad de Chagas en las Américas leída en esta sesión.

Como delegado oficial del Ecuador y especialmente como un de los 400.000 habitantes de la ciudad de Guayaquil le expreso mi más absoluta solidaridad a sus conceptos, ideas y sugestiones.

Sin considerar el problema en general de la enfermedad en mi país, solo quiero hacer conecer algunos datos relacionados con nuestras investigaciones en el transcurso de estos 2 últimos años practicadas en la ciudad de Guayaquil, pués debo hacer especial énfasis en la característica que adopta esta endemía entre nosotros, de ser urbana y no rural, siendo Guayaquil, el puerto principal de mi país, el más grande foco. Este hecho importante ha creado un complejo problema sanitario, pues de vez en cuando, a propósito de un caso agudo en algún ciudadano de una mejor esfera social, se desata una ola de protesto ciudadano por la falta de alguna acción profiláctica.

Los datos son los siguientes:

Casos agudos diagnosticados cuya procedencia es Guayaquil, 86.

*Triatomas dimidiates* capturados 465, de los cuales el 53,5 por ciento estaban infectados.

Reacciones de fijación del complemento practicadas en total entre la población, 1.540, de las cuales el 19,2 por ciento eran positivas

En un próximo Congreso de Medicina a reunirse en Guayaquil en el próximo octubre seran aportadas las primeras 100 cardiopatias agudas de etiología chagásica.

El año de 1950 el Doctor Juan Montalván inició un plan piloto en la ciudad de profilaxis utilizando gamexanno con el mejor de los

éxitos, pues la población triatomídea de las marganas tratadas se redujo a 0. Lamentablemente esta valiosa experiencia no pudo ser continuada por falta de fondos. El año pasado, con miras a erradicar este grave problema médico-social en la ciudad, se solicitó la cooperación de la Oficina Sanitaria Panamericana, la que informó no haber posibilidades económicas por el momento.

Insisto en que la elaboración de un plan de erradicación de Guayaquil es impostergable.

Doctor Luis Espinoza (Ecuador) — El Prof. Del Ponte señala distintos vertebrados como los que mantienen la fuente de infección como reservorios del *Tripanosoma cruzi*.

En mis estudios sobre los reservorios en Guayaquil encontré que el *Didepplis azorae* o *paraqüayensis*, llamado entre nosotros *raposa* o *zorro*, era el principal reservorio del parásito. Examiné 104 ejemplares, de los cuales el 44 por ciento de ellos tenian el parásito en su sangre periférico.

Este reservorio vive en forma semidoméstica en las habitaciones de la ciudad y nosotros atribuímos el elevado índice de infestación de los triatomídeos a su convivencia con estos reservorios. Un hecho mui importante es que estos animales presentan una parasitemia constante, lo que no acontece con los animales domesticos conocidos, como el perro, gato, rata, en los que es periódico.

Dr. Fernando M. de Bustamante (Brasil) — Quero fazer dois rápidos comentários sobre o trabalho aqui apresentado pelo Dr. Emmanuel Dias.

O primeiro refere-se à erradicação do *T. infestans* com apenas duas aplicações de B. H. C. Sinto-me feliz pelo facto de o Dr. Dias ter verificado essa possibilidade, pois a mesma já fora por nós demonstrada em 1952-1953, conforme trabalho publicado, em 1954, na *Revista Brasileira de Malariologia e Doenças Tropicais*, vol. VI, n.º 1, quando então escrevemos: «O problema da erradicação do *T. infestans* de uma área infestada é um problema tècnicamente superado. Sendo uma espécie eminentemente doméstica, a sua eliminação das casas significará, certamente, sua erradicação da área infestada.»

Penso, ademais, que o intervalo de um mês entre as duas aplicações de B. H. C., recomendado pelo Dr. Dias, não é condição obri-

gatória ou indispensável, pois em nossa experiência a erradicação do *T. infestans* foi conseguida com duas aplicações feitas com intervalo de 4 meses e até de 6 meses. Em 1957 fizemos cuidadosa revisão em todas as casas da área, situada no estado do Rio de Janeiro, com resultado negativo, pois nenhum exemplar de triatomíneo foi encontrado.

Meu segundo comentário refere-se à possibilidade de erradicação dos triatomíneos em geral, admitida pelo Dr. Emmanuel Dias.

Para espécies semi-silvestres, como são a maioria das espécies transmissoras da doença de Chagas, a eliminação dos triatomíneos das casas pode não significar a sua erradicação da área, aquelas podendo ser reinvadidas após a interrupção do insecticida. Em minha opinião, por conseguinte, para essas espécies não estritamente domésticas, não se deve falar em erradicação com insecticidas aplicados nas habitações.

Dr. Durval Lucena (Brasil) — *To Dr. Goble:* What is the explanation for the fact that in the United States, were there are triatome bugs infected, reservoirs and two human acute cases had been reported, the disease is, at present day, practically inexistent?

What happened to explain this fact that the race (or races) of tripanosome is not pathogenic to humans?

Dr. Francisco J. Aguilar (Guatemala) — En relación al trabajo del Dr. Lynz, quién señaló el *Triatoma dimidiata* y el *Rhodnius prolixus,* hay que agregar el *Triatoma nitida* y probablemente el *Triatoma picturata.*

Como reservorios se han encontrado infestados naturalmente: el perro, armadillo, tocuazín *(Dydelphus)* y mapache *(Procyon lotor).*

En la Sección de Trypanosomiasis del Instituto de Enfermedades Tropicales Rodolfo Robles, de la Dirección General de Sanidad Pública, hay revisados 650 casos de enfermedad de Chagas, diagnosticados por examen de gota gruesa, hemocultivos, xenodiagnóstico y reacción de Machado Guerreiro (ésta última se practica desde 1953).

Lamento que en esta Sección del IV Congreso Internacional de Medicina Tropical no haya habido comunicaciones centroamericanas, ya que: en 1913 el Dr. Segovia (San Salvador) diagnosticó la E. de Chagas; en Costa Rica hay trabajos muy completos de Zeledón; en

El Salvador, los recientes trabajos del Dr. Peñalver y la Dr.ª Cabezas. En Guatemala, los trabajos de Reichenow (1932), de León (1932), diagnosticando más de 20 casos, y de Peñalver y colaboradores (1952--1958) elevan el número de casos a más de 650.

Dr. Swellengrebel (Netherlands) — En presencia de tan gran nombre de expertos quisiera saber cual es el nombre exacto del organismo: *Trypanosoma cruzi* o *Schizotrypanum cruzi?*

Dr. A. Neghme (Chile) — Recalcando la importancia médico--social de la enfermedad de Chagas, se refiere a la demonstración que se ha hecho en Chile de varios casos de transmisión congénita en madres infectadas crónicas sin sintomatología clínica (casuística del Prof. Ariztía y colaboradores). Estos casos de infección congénita podrían ser relativamente frecuentes en areas rurales de la endemia chagásica.

Dr. Felix Pifano (Venezuela) — En Venezuela, desde que la enfermedad de Chagas fué comprobada por Tejera, se investigado en dos aspectos: (1) desde el punto de vista ecológico y (2) en lo aspecto cardiopatico.

No se ha hecho una evaluación del total de casos de enfermedad de Chagas en el país. Es frecuente, sin embargo, la miocardiopatia y las investigaciones de laboratorio demuestran su etiología prevalentemente chagásica. Investigaciones electrocardiograficas sobre personas de áreas endémicas de enfermedad de Chagas, especialmente por debajo de 16 años, demuestran en los casos infectados la existencia de daños miocardicos en el 50 por ciento de los casos (bloqueo).

The meeting was opened by the Chairman, Prof. Carlos P. Trincão, the Rapporteurs Dr. Andrade e Silva and Dr. Emmanuel Dias being present; the Secretary was Dr. Augusto R. Pinto.

The following papers were presented and discussed.

# CHIMIOTHÉRAPIE ET CHIMIOPROPHYLAXIE DE LA MALADIE DU SOMMEIL À *T. GAMBIENSE*

G. NEUJEAN

## I — CONSIDÉRATIONS GÉNÉRALES

Il semblerait que le déclin de la trypanosomiase à *T. gambiense*, devant les résultats obtenus par la prophilaxie chimique, réduise de beaucoup les problèmes que pose le traitement de cette maladie. En réalité, la réduction massive du réservoir de virus sous l'effet de la prophylaxie chimique a en comme résultat de déplacer le problème thérapeutique du plan collectif ou social sur un plan de médecine individuelle ou de médecine de cas. Nous cherchions jadis par une thérapeutique de masse, forcément schématique, à «blanchir le malade», c'est-à-dire à l'empêcher d'être infectieux pour la glossine, tout autant qu'à le guérir au sens thérapeutique du mot. Actuellement, qu'un tel résultat s'obtient plus efficacement par la prophylaxie chimique, il ne peut plus nous suffire de blanchir parasitologiquement parlant les trypanosomés, mais il nous appartient de rechercher pour chaque cas la guérison au sens complet de ce terme. Ce programme implique évidemment des observations et des investigations cliniques approfondies, ainsi qu'une connaissance précise des ressources thérapeutiques dont nous disposons actuellement.

Dans le traitement des trypanosomiases humaines, le but primordial à atteindre est la destruction du protozoaire. Les thérapeutiques

non spécifiques peuvent être d'une certaine utilité, soit lors d'épisodes aigus, soit chez de vieux cas où la maladie, non soignée, a fini par toucher toutes les fonctions physiologiques, soit enfin pour améliorer les séquelles nerveuses qui peuvent subsister après guérison parasitologique. L'arsenal thérapeutique dont nous disposons est abondant, mais, malgré l'aide que ces médications non spécifiques peuvent apporter, nous ne saurions assez rappeler que *l'action thérapeutique doit avant tout être centrée sur la destruction du protozoaire* [1].

L'expérience a fait ressortir deux données de base qui, pour être élémentaires, n'en méritent pas moins de devoir être rappelées au début de cet exposé: 1) c'est au début de la maladie que les chances de guérison sont les plus grandes; 2) lorsque la thérapeutique d'attaque a échoué, quel que soit l'état d'avancement de la maladie, le pronostic en est fortement assombri et la reprise du traitement pose un probème toujours difficile, souvent angoissant à résoudre.

Le choix du traitement d'attaque de tout trypanosomé est donc d'importance capitale. Les malades seront l'objet d'une étude biologique minutieuse. La maladie du sommeil à *T. gambiense* est une méningo-encéphalite qui se développe après une période lymphatico-sanguine initiale plus ou moins longue, dont la durée ne peut être évaluée et varie probablement suivant la souche en cause et certaines résistances individuelles [2]. L'appréciation de ces périodes est essentielle pour le choix des médicaments spécifiques, car certains de ceux-ci (que nous appellerons *trypanocides périphériques*) sont inactifs au delà de la barrière hémato-encéphalique. Elle est très délicate pendant la période transitoire qui marque le passage du stade lymphatico-sanguin au stade nerveux et ne pourra être basée que sur un examen approfondi du liquide céphalo-rachidien [3]. Hormis des cas spéciaux

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[1] Nous n'ignorons pas qu'il existe des formes silencieuses (porteurs sains ou états latents), ce qui n'infirme en rien le principe que nous venons de rappeler, car, à l'heure actuelle, nous ignorons tout de l'avenir de ces formes silencieuses.

[2] La clinique a permis d'observer tous les intermédiaires entre les cas «classiques» dus à *T. gambiense* et ceux dus à *T. rhodesiense*.

[3] On se rappellera les données suivantes: parmi les malades dont la leucocytose rachidienne ne dépasse pas 3 cellules, 15 % ont déjà une albuminorachie supérieure à 25 cg °/₀₀ et 30 % une leucocytose céphalique trop élevée. Parmi les malades dont le liquide rachidien montre une leucocytose ne dépassant pas 3 cellules et l'albuminorachie 25 cg °/₀₀, il en est 23 % dont la réaction du benjoin colloïdal est

qui requièrent l'intervention urgente du thérapeute (épisodes mentaux ou atteinte profonde de l'état général), *le médecin doit prendre le temps nécessaire pour procéder à ces recherches et éviter à tout prix d'administrer aucun trypanocide tant que le bilan biologique du malade n'a pas été établi avec certitude.*

## II — APERÇU SUR LES TRYPANOCIDES ANCIENS ET NOUVEAUX

Nous ne reviendrons pas sur l'historique des arsenicaux. Il est connu de tous. En 1939, Findlay, faisant le point de la question, pouvait écrire:

> «Le nombre de produits actuellement employés contre les trypanosomiases humaines et animales est relativement réduit: la tryparsamide et l'orsanine parmi les arsenicaux pentavalents, la germanine et, chez l'animal, le surfen C. Même ces quelques produits présentent de sérieux inconvénients. La valeur des arsenicaux pentavalents reste discutable à la première période et expose à l'atrophie du nerf optique dans les cas avancés, tandis que la germanine est sans action lorsque le système nerveux est entrepris.»

Nous ajouterons que l'émétique de potasse avait donné occasionnellement de bons résultats, notamment au Congo Belge. En revanche, les cas de résistance à la tryparsamide se multipliaient.

Examinons maintenant les trypanocides qui furent proposés dans les 10 années qui suivirent et dont la valeur peut être actuellement appréciée avec un recul suffisant.

Parmi les arsenicaux pentavalents, sont à retenir les dérivés de l'acide triazinarsonique ou melarsen (Friedheim), dont le mel B ou

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anormale. On doit donc s'attendre à ce que parmi ces malades à liquide apparemment normal quant à la leucocytose et à l'albuminorachie, un quart environ montre déjà des signes de réaction méningée (qui peuvent bien entendu avoir une autre cause que la trypanosomiase). C'est ce que l'expérience confirme par les échecs du Bayer 205 et de la pentamidine chez les malades que l'on estime être en période lymphatico-sanguine.

arsobal est la forme la plus maniable. Les autres arsenicaux proposés, comme le butarsen, se sont montrés nettement inférieurs.

Parmi les trypanocides non métalliques, certaines diamidines aromatiques se sont montrées remarquablement actives contre les trypanosomes périphériques. Ce sont les sels du diamidinodiphenoxypentane qui se sont finalement imposés, à cause de leur faible toxicité (pentamidine, lomidine). Ces produits ont en outre des propriétés prophylactiques remarquables qui en font l'arme actuellement la plus puissante dans la lutte contre la maladie du sommeil.

Il résulte de l'expérience acquise que l'on peut retenir comme:

1) Très actif à tous les stades de la maladie: l'arsobal.
2) Médiocre à la première période et actif en période nerveuse (en dehors des cas de chimio-résistance): la tryparsamide.
3) Actifs uniquement à la phase lymphatico-sanguine: le Bayer 205 et la pentamidine (lomidine).

À la phase lymphatico-sanguine, les trypanocides non métalliques gardent la préférence des cliniciens à cause de leur plus grande maniabilité.

Seuls, les organo-métalliques (arsenic et antimoine) ont pu jusqu'à présent guérir la trypanosomiase à *T. gambiense* à son stade de méningo-encéphalite. La tryparsamide a perdu considérablement de sa valeur par suite de l'apparition en certaines régions d'une chimio-résistance qui s'est rapidement généralisée. Elle reste néanmoins un stabilisant des altérations nerveuses qui peut encore rendre de grands services. L'apparition de la chimio-résistance à la tryparsamide semble liée à l'utilisation massive de ce produit et avant elle d'autres arsenicaux, comme l'atoxyl. Ce fait ne doit jamais être perdu de vue lorsque nous traitons des porteurs de trypanosomes et que nous nous proposons de leur administrer un arsenical. En cas d'échec, les souches de trypanosomes provenant de ces malades risquent fort de montrer une chimio-résistance progressive. Les nouveaux produits (arsobal et pentamidine) ont momentanément fait passer les antimoniés au second plan. Il n'empêche que ceux-ci peuvent encore rendre des services et doivent certainement être retenus, ne fut-ce que comme base de départ pour des recherches futures. Un antimonié proposé par Frie-

dheim et calqué sur la formule de l'arsobal, le MSbB, présente un intérêt certain.

Il serait téméraire de vouloir faire une comparaison chiffrée de l'activité des trypanocides qui sont actuellement retenus dans la pratique, car il ne pourrait être tenu compte dans une statistique générale de toute une série d'éléments qui tiennent tant du malade que des souches en cause. Nous exposerons à titre d'information les résultats globaux que nous avons obtenus en employant un seul trypanocide sur 743 nouveaux cas, représentant tous les stades de la maladie. Nous avons éliminé du relevé des évolutions favorables les cas qui avaient moins de 6 mois de recul et avons — pour les présenter dans les deux tableaux 2 et 3 — classé les cas d'après l'importance des altérations du liquide céphalo-rachidien. Le premier montre les réussites; le second, les décès imputables à la thérapeutique.

Les malades sont classés en 4 catégories ainsi définies, d'après les résultats de l'analyse du liquide céphalo-rachidien:

TABLEAU 1

| Catégories | L'albuminorachie | Leucocytose de |
|---|---|---|
| 1ère catégorie. . . . . | Ne dépasse pas 25 cg $^0/_{00}$ | Moins de . . 3 cellules |
| 2ème catégorie. . . . . | Ne dépasse pas 25 cg $^0/_{00}$ | Moins de . . 3 cellules |
| 3ème catégorie. . . . . | Dépasse. . . . 25 cg $^0/_{00}$ | Plus de . . . 100 cellules |
| 4ème catégorie. . . . . | Dépasse. . . . 25 cg $^0/_{00}$ | Plus de . . . 100 cellules |

TABLEAU 2

| État d'avancement de la maladie | Pourcentage de guérisons ou évolutions favorables après | | | |
|---|---|---|---|---|
| | Bayer 205 | Pentamidine | Tryparsamide | Arsobal |
| 1ère catégorie . . . . . | 76,2 | 90 | — | 96,2 |
| 2ème catégorie . . . . . | 66 | 79 | 41,3 | 94,6 |
| 3ème catégorie . . . . . | 44,5 | 66 | 50 | 88,6 |
| 4ème catégorie . . . . . | — | — | 41,8 | 72,9 |

Nous utilisons le terme de catégorie afin de ne pas préjuger des mécanismes pathologiques. Cette classification doit servir uniquement à grouper les cas qui présentent le plus d'analogie suivant un schéma correspondant plus ou moins à l'état d'avancement de la maladie.

Ces tableaux montrent à suffisance que, quel que soit le trypanocide utilisé, avec l'évolution de la maladie vers la méningo-encéphalite, 1) *les chances de guérison vont en diminuant* et 2) *les dangers de la thérapeutique vont en augmentant.*

Ils démontrent aussi la supériorité de l'arsobal à toutes les périodes de la maladie et la valeur de la pentamidine à la période lymphatico-sanguine.

TABLEAU 3

| État d'avancement de la maladie | Pourcentage de décès après | | |
|---|---|---|---|
| | Pentamidine | Tryparsamide | Arsobal |
| 1ère catégorie . . . . . | 0 | — | 3,8 * |
| 2ème catégorie . . . . . | 1,8 | 0 | 0,7 |
| 3ème catégorie . . . . . | 0 | 12,5 | 5,7 |
| 4ème catégorie . . . . . | — | 16,3 | 7 |
| Pourcentage général de décès . . . . . | 0,75 | 8,3 | 3,2 |

* Ce chiffre est nettement trop élevé car il est grevé par les accidents toxiques qui ne bénéficiaient pas encore du traitement au B.A.L.

## III — SCHÉMAS DE TRAITEMENT PROPOSÉS POUR LES NOUVEAUX CAS

La conférence de Salisbury sur les trypanosomiases (1956) a recommandé des schémas de traitement auxquels il est utile de se référer. Aussi, notre exposé sera surtout un rappel critique des idées qui y ont été développées et des conclusions adoptées.

### 1. PÉRIODE LYMPHATICO-SANGUINE.

Nous établirons comme prémisses que l'utilisation du Bayer 205 et de la pentamidine ne peut se justifier que si *l'on est certain que la période lymphatico-sanguine n'est pas dépassée.* Face aux arsenicaux

malgré tout plus dangereux, le médecin reste tenté, à juste titre, de les utiliser.

a) *La pentamidine* (lomidine) a nettement la préférence aussi bien sur le plan de l'activité que celui de la toxicité ou de la facilité d'administration.

Si le liquide rachidien contient moins de 25 cg pour mille d'albumines et moins de 3 cellules et si la réaction au benjoin colloïdal est normale, on est parfaitement autorisé à utiliser la pentamidine. Il sera procédé à une ou deux cures comportant au total 6 à 10 injections *intramusculaires* quotidiennes d'un sel de diamidinodiphénoxypentane à raison de 3 mg à 4 mg base/kg sans que la cure totale dépasse jamais 40 mg /kg [1]. Seule l'action hypotensive est à craindre et peut être facilement contrôlée. L'observation du malade n'est requise que dans les heures qui suivent immédiatement les injections. Notre préférence va aux cures de 10 injections quotidiennes de 3 mg base/kg. La grossesse n'est pas une contre-indication.

Il n'est peut-être pas inutile de rappeler que, comme pour tous les produits thérapeutiques, en cas de préparation sur place des solutions injectables, l'eau utilisée doit offrir toutes les garanties désirables de pureté et de stérilité et qu'en principe les lots préparés extemporanément ne se conservent pas et peuvent, en climat chaud, devenir rapidement dangereux [2].

b) *Le Bayer 205* (germanine, moranyl, suramin ou antrypol) reste une des gloires de la synthèse en matière de chimiothérapie et garde une valeur indéniable. Ainsi que nous l'avons montré, il est un trypanocide périphérique puissant. Lorsque la pentamidine est formellement contre-indiquée, on pourra donc y recourir. Ses inconvénients

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[1] Rappelons les équivalents base des 3 sels utilisés:

1) 1 g dichlorhydrate . . . = 8,83 g base.
2) 1 g diméthanesulfonate = 0,64 g base.
3) 1 g iséthionate . . . . . = 0,57 g base.

et inversement

1 g base . . . . . = 1,21 g dichlorhydrate.
= 1,56 g diméthanesulfonate.
= 1,74 g iséthionate.

[2] Il existe des solutions commercialisées qui donnent toute satisfaction et permettent d'éviter les risques que nous venons de signaler.

sont bien connus: albuminurie momentanée parfois impressionnante et généralement sans gravité, réactions cutanéo-muqueuses rarement sérieuses. Une cure comportera 5 à 6 injections intraveineuses à raison d'une ou deux par semaine de façon à atteindre un total de 200 mg/kg pour la cure. On fera boire le malade abondamment et on lui imposera le repos.

Malgré que le Bayer 205 soit utilisé depuis une trentaine d'années, il semble bien que sa valeur n'a pas beaucoup diminué. Les craintes de voir apparaître une chimio-résistance spécifique des trypanosomes touchés par le médicament ne se sont pas réalisées, contrairement à ce que l'on a observé avec les arsenicaux.

c) *Association Bayer 205 (pentamidine).* — L'interaction pharmacologique (Lourie) de ces deux produits a amené les chercheurs à les essayer soit en association soit en combinaison (moranylate de lomidine, produit insoluble dans l'eau et pouvant être administré *per os*).

Nous n'avons essayé, pour notre part, que l'association des produits habituels, administrés séparément, le premier par voie intraveineuse, le second en intramusculaire.

Le schéma de la cure a comporté des séries hebdomadaires:

1er jour: 1 g à 2 g Bayer 205 + 4 mg base/kg pentamidine.
3ème jour: 4 mg base/kg pentamidine.
5ème jour: 4 mg base/kg pentamidine.

Nous avons administré jusqu'à 4 cures en laissant un repos de 15 jours entre la 2ème et la 3ème séries.

Cette association a été très bien supportée et il semble donc bien que le Bayer 205 ait permis au malade de supporter des doses de pentamidine qui autrement auraient déclenché des accidents toxiques, voire mortels. Nos essais ont porté sur 38 cas. Les signes toxiques provoqués par le Bayer 205 et spécialement la desquamation épidermique et muqueuse persistent; l'albuminurie n'est pas plus fréquente. En conclusion, nous considérons que les essais avec cette association méritent d'être continués. Elle doit permettre, semble-t-il, un traitement d'attaque puissant dans les cas où le système nerveux n'est pas encore atteint.

d) *Les arsenicaux.* — La tryparsamide est à rejeter. L'arsobal, en revanche, est le médicament qui donne les meilleurs résultats, avec

un risque d'accidents mortels de 3 pour cent environ que l'on ne peut ignorer. Hormis ce risque, une injection unique de 4 mg/kg assure la guérison du malade en période lymphatico-sanguine. Nous reviendrons plus loin sur son emploi.

2. Période de réaction méningée (période intermédiaire).

Dès que celle-ci se manifeste, les chances de guérison avec les trypanocides non métalliques se réduisent rapidement, même si on en augmente les dosages. L'évolution vers la méningo-encéphalite n'est pas arrêtée quoique les trypanosomes disparaissent momentanément de la circulation périphérique. Il faudra donc adopter comme ligne de conduite que si le malade montre les moindres signes d'irritation méningée (et ceux-ci ne sont décelables que par l'analyse du liquide céphalo-rachidien), il faudra passer aux arsenicaux.

3. Période de méningo-encéphalite.

Il existe encore des régions où la chimio-résistance à la tryparsamide ne s'est pas encore installée. Dans ce cas on pourra recourir à ce médicament, qui est plus maniable que l'arsobal. Autrement, ce dernier sera notre seule et ultime ressource [1].

a) *Tryparsamide.* — Ce produit est familier de tous ceux qui s'occupent de la maladie du sommeil. Les cures classiques comportent 10 à 15 injections intraveineuses, à raison d'une injection par semaine, de 30 à 40 mg/kg. En général, on ne dépassera pas 2 g à 2,5 g chez l'adulte. Lorsque la solution est correctement préparée avec un produit de bonne qualité, les risques sont réduits, sauf chez les malades anciens à mauvais état général. Chez ces derniers, on peut observer d'une part, dès la première injection, des réactions violentes, type Herxheimer, et il n'est pas rare que la mort s'ensuive rapidement. D'autre part, une chute progressive de la vue peut apparaître après la 3ème ou la 4ème injection, parfois même avant, traduisant l'atteinte

[1] Nous croyons utile de rappeler qu'il ne paraît pas indiqué d'injecter trop rapidement après une ponction lombaire les médicaments qui franchissent la barrière hémato-encéphalique.

du nerf optique. Il semble bien, vu le peu d'efficacité du B. A. L. sur ces accidents, que la trypanosomiase elle-même y joue un rôle aussi important que l'arsenic. Ces accidents peuvent survenir plus exceptionnellement chez des malades récents. Qu'il s'agisse de la réaction d'Herxheimer ou de l'atteinte du nerf optique, ces accidents sont imprévisibles. Ils sont plus fréquents et plus graves dans les cas avancés, et nos moyens d'y pallier sont très limités.

En revanche, chez la plupart des malades, la tryparsamide est fort bien tolérée et les cures peuvent être multipliées sans danger. Elle améliore l'état général et même lorsqu'elle n'a plus d'action spécifique, elle bloque parfois longtemps encore l'évolution nerveuse. Nous ne possédons pas de test réellement valable qui permette de juger, pour un cas déterminé, de la chimiorésistance de la souche en cause.

*b*) *Arsobal.* — Malgré la moindre sécurité qu'il paraissait offrir par rapport aux autres trypanocides et après plusieurs années de tâtonnements, ce médicament s'est imposé. En effet, par son activité à tous les stades de la maladie, l'arsobal les surclasse tous.

L'expérience nous a maintenant appris à l'utiliser avec un minimum de risques si on respecte certaines précautions que nous résumerons. Le malade doit rester sous observation médicale, il doit être préparé (repos et diète), le matériel d'injection doit être rigoureusement sec. Le malade sera gardé sous surveillance constante pendant la journée qui suit l'injection. Le régime sera léger et l'alcool sévèrement prohibé. Les risques de l'arsobal sont ceux bien connus de l'intoxication arsenicale. La sensibilité individuelle du malade joue un rôle plus important que le dosage utilisé. La plupart des accidents toxiques apparaissent après la 1ère ou la 2ème injection. Très souvent impressionnants, il faut distinguer ceux qui cèdent presque toujours d'eux-mêmes, les plus fréquents, et les accidents graves souvent mortels. Les premiers consistent en tremblements, vertiges, vomissements, douleurs abdominales, arthralgies et myalgies (rhumatisme arsenical), ataxie, dysarthrie, dermatites arsenicales (érythrodermie), etc., qui cèdent le plus souvent spontanément et dont on peut, si le cas le requiert, hâter la disparition par administration de B. A. L. (dimercaptopropanol). Quant à l'accident grave par excellence, c'est l'encéphalopathie arsenicale qui peut se manifester brutalement, même après une seule injection ; elle se caractérise par des crises convulsives, de

l'hyperthermie, un faciès vultueux et finalement le coma et la mort si on n'intervient rapidement par une cure prolongée de B. A. L.[1]

Actuellement, on peut estimer à 7 pour cent les intoxications diverses et à 3 pour cent la mortalité imputables au médicament. Il n'est pas douteux qu'avec une meilleure connaissance de son emploi ces chiffres ne s'amélioreront encore.

On tiendra compte pour choisir le schéma de cure à appliquer de deux données imposées par l'expérience: la première concerne la dose elle-même, la seconde concerne le rythme à adopter pour les injections.

1) Il ressort des essais pratiqués qu'en dessous de 3,6 mg/kg le nombre des échecs va rapidement croissant et nous estimons, avec Friedheim, que cette dose ne peut jamais être abaissée, et nous ajouterons même, surtout lors de la première injection. Vouloir tâter la sensibilité du malade, c'est l'exposer à l'échec final, dont il est inutile de souligner la conséquence souvent fatale. Ce qui nous renforce dans notre point de vue c'est que d'une part nous avons injecté à de nombreux malades 4 mg/kg sans aucun ennui et que par ailleurs, les premières injections ayant été tolérées, nous avons administré des cures comportant jusqu'à 30 injections de 3,6 mg/kg du produit sans accident. Nous répétons que ce qui est redoutable est bien plus la sensibilité du malade au produit que la quantité injectée de celui-ci.

2) Pour ce qui est du rythme à adopter, on retiendra que les tentatives faites pour traiter les malades par injections espacées, par exemple à raison de deux fois par semaine, se sont soldées par des échecs. On peut actuellement recommander les cures de 3 à 4 injections quotidiennes de 3,6 mg/kg. Sauf incident, ces cures seront répétées deux ou trois fois, en les espaçant d'un repos de une ou deux semaines. Il reste des hésitations quant au nombre de cures à administrer. Celui-ci ne peut être évalué que d'après la gravité du cas, l'importance de l'atteinte des centres nerveux, l'état général et le comportement du malade au cours du traitement et enfin d'après la résistance de la souche responsable, donnée souvent difficile à évaluer pour un cas déterminé. Les chiffres que nous donne l'examen du liquide céphalo-rachidien traduisent davantage un état réactionnel

[1] Rappelons que celui-ci s'administre par voie intramusculaire à raison de 1,5 $cm^3$ toutes les 3 heures.

qu'un état pathologique. Aussi, en sommes-nous réduits à nous baser sur l'expérience clinique. Il ressort de celle-ci qu'un malade ne tirera guère de bénéfice d'un traitement qui comportera plus de 8 à 12 injections.

Nous traiterons des associations thérapeutiques et des cures de sécurité au chapitre suivant.

## IV — LE PROBLÈME DE LA RECHUTE ET DE SON TRAITEMENT

Ce problème en implique un autre qui est celui de la guérison. Nous utilisons le mot de *rechute* pour suivre une tradition. Ce terme implique une période de guérison intermédiaire, ce qui n'est pas exact. Il s'agit de la reprise d'une évolution momentanément arrêtée.

La réussite ou l'échec d'un traitement d'attaque sont souvent difficiles à apprécier dans les premiers mois qui suivent celui-ci. On s'appuiera sur l'observation clinique et surtout sur les résultats des analyses. La disparition définitive du protozoaire est une signature de guérison lorsque, après multiples recherches, elle est constatée pendant un temps suffisamment long. Il faut toutefois prendre en considération que dans de nombreux cas, même traités à la période lymphatico-sanguine, l'échec du traitement n'est pas décelé par la présence du protozoaire, mais bien par l'image fournie par l'analyse du liquide céphalo-rachidien. Le contrôle des malades traités doit donc comporter, outre la recherche répétée du trypanosome, un examen minutieux et périodique du liquide céphalo-rachidien [1].

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[1] Lorsque le malade traité était au stade nerveux, la normalisation du liquide céphalo-rachidien se réalise lentement. Toutefois, au bout de 6 mois, si le cas évolue favorablement, l'amélioration doit être franche, surtout en ce qui concerne l'albuminorachie.

Si le traitement échoue, on observera généralement endéans ces 6 premiers mois l'apparition ou l'augmentation des altérations. On se souviendra qu'après administration d'arsobal, on observe souvent une réaction méningée momentanée d'orgine chimique. Celle-ci est sans gravité spéciale, mais enlève toute valeur aux données fournies par l'analyse du liquide céphalo-rachidien pendant les 6 à 8 semaines qui suivent la cure. Dans la pratique, le sang sera examiné aussi souvent que possible et le liquide céphalo-rachidien sera analysé soigneusement au bout du 3ème et du 6ème mois qui suivent la cure d'attaque. Ultérieurement, le rythme des examens du liquide céphalo-rachidien pourra être espacé si les résultats sont nettement favora-

A. — Principes généraux de traitement.

Nous envisagerons successivement les situations qui peuvent se présenter:

1 — Malade à la période lymphatico-sanguine faisant une rechute après traitement d'attaque avec un trypanocide périphérique (Bayer 205 ou pentamidine):

*a*) Dans cette éventualité, si l'examen clinique est complètement négatif et si le liquide céphalo-rachidien est resté normal, on peut envisager de reprendre le traitement avec un trypanocide périphérique, éventuellement en passant de l'un à l'autre. C'est ainsi que des échecs après Bayer 205 ont guéri après un traitement à la pentamidine. Personnellement, nous ne pouvons conseiller cette façon de faire, qui doit être réservée à des cas particuliers (mauvais état général, maladies intercurrentes, etc.).

*b*) Si le liquide céphalo-rachidien montre des altérations, il n'y a pas de problème thérapeutique. Il faut passer aux arsenicaux et de préférence à l'arsobal.

2 — Malades en période lymphatico-sanguine faisant une rechute après traitement d'attaque par un arsenical: un seul traitement s'impose: l'arsobal. Il faut considérer la situation comme grave et instituer un traitement intensif, quelle que soit l'importance des altérations du liquide céphalo-rachidien.

3 — Malades au stade de méningo-encéphalite (ou d'irritation méningée) faisant une rechute: en principe ces malades ont dû être traités précédemment avec un arsenical. Que ce soit avec la tryparsamide ou avec l'arsobal, la reprise du traitement avec l'arsobal s'impose. Le traitement sera intensif et éventuellement répété, si le malade le supporte bien, même si les éléments biologiques d'appréciation sont des plus satisfaisants. On se souviendra que la présence de trypanosomes après une cure d'arsobal n'exclut pas une réussite ultérieure avec de nouvelles cures d'arsobal. Les chances de guérison des malades chez lesquels le traitement d'attaque a échoué et que l'on soumet à un traitement à l'arsobal sont de:

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bles. Si l'albuminorachie ne cède pas, il y a lieu de rester très attentif et de ne pas hésiter à répéter les ponctions lombaires.

90 pour cent pour ceux qui ont été traités au Bayer 205 ou à la pentamidine.
88 pour cent pour ceux qui ont été traités à la tryparsamide.
50 pour cent pour ceux qui ont été traités à l'arsobal.

B — Remarques diverses.

1 — Il ressort de cet exposé que l'échec d'un médicament doit faire rechercher autant que possible une autre thérapeutique. La pentamidine guérit des cas qui ont résisté au Bayer 205. L'arsobal guérit des cas qui ont résisté à la tryparsamide. Il est vrai aussi que exceptionnellement le traitement des rechutes par le même médicament peut aboutir à la guérison, que ce soit avec la tryparsamide ou avec l'arsobal. De ce qui précède, nous retiendrons qu'il est essentiel de rechercher la guérison par le traitement d'attaque, ainsi que nous l'avons dit au début de cet exposé.

2 — En cas d'échec après traitement de la rechute, le problème thérapeutique devient particulièrement ardu et les chances de guérison du malade se réduisent considérablement. Il n'est pas possible de fixer une ligne de conduite à ce stade. Après échecs successifs à la tryparsamide, l'émétique peut rendre des services. Après l'arsobal, nous en sommes moins certains. Toutes les tentatives sont permises et nous signalons les espoirs que l'on peut mettre en la nitrafurazone (furacine).

C — Associations thérapeutiques.

Le principe même de l'association d'un trypanocide périphérique à un arsenical peut se défendre car on peut espérer de la sorte une stérilisation plus rapide du réservoir périphérique afin d'empêcher la réalimentation des centres nerveux par de nouvelles pénétrations de trypanosomes. Cet argument est peut-être valable pour la tryparsamide, qui est un mauvais trypanocide périphérique, mais l'est beaucoup moins pour l'arsobal.

Un fait pratique est à retenir. L'administration simultanée de trypanocides est souvent dangereuse et ne peut guère être recommandée même si les résultats enregistrés sont parfois spectaculaires.

En revanche, nous estimons comme tout à fait défendable d'encadrer une cure arsenicale par l'administration à doses modérées d'un trypanocide périphérique. À notre connaissance, il n'y a pas suffisamment d'observations relatées pour pouvoir recommander l'un ou l'autre schéma thérapeutique.

### D — Cures de sécurité.

On intitule de la sorte l'administration d'un trypanocide périphérique après traitement du malade. Cette façon de faire poursuit deux buts. Le premier a été exposé dans le chapitre précédent sur les associations thérapeutiques et vise à arrêter l'invasion des centres nerveux. Le second cherche, en cas d'échec thérapeutique, à supprimer ou au moins à réduire les trypanosomes périphériques et à empêcher le malade d'être dangereux pour la collectivité. Cette façon de voir est défendable, même si dans les cas passant à chronicité les trypanosomes périphériques se montrent de plus en plus rares et deviennent de moins en moins transmissibles. On pourra administrer périodiquement de la pentamidine, sans espoir toutefois d'améliorer le malade.

## V — LA PROPHYLAXIE CHIMIQUE DE LA TRYPANOSOMIASE À *T. GAMBIENSE*

Trois produits, le Bayer 205, la pentamidine et le MSbB, ont été proposés, dont deux, le Bayer 205 et la pentamidine, ont été essayés à plus ou moins grande échelle.

C'est avec le Bayer 205 que fut entrevue la possibilité de protéger les individus contre la piqûre infectieuse de la glossine. La protection conférée par ce produit ne dépasse pas 3 mois, et est de plus courte durée chez de nombreux individus. L'administration intraveineuse rend difficile la généralisation du procédé aux collectivités menacées.

Avec la pentamidine, la prophylaxie chimique allait connaître un succès sans précédent. En effet, si on se place du point de vue de l'importance du virus circulant, les résultats obtenus suite à des

campagnes répétées de pentamidinisation sont extraordinaires [1]. Dans les régions où les indices permanents de nouvelle infection restaient bloqués au dessus de 1 pour cent, la prophylaxie chimique les a réduit à des chiffres aussi bas que 0,1 à 0,01 pour cent. L'amenuisement des chiffres basés sur le dépistage des cas de plus en plus sporadiques empêche actuellement en maints endroits l'établissement des cartes d'endémie suivant la méthode habituelle, qui indiquait l'importance des indices régionaux. On ne peut plus, dans les régions protégées par la prophylaxie chimique, établir qu'une carte de présence du virus [2].

Actuellement, il s'agit donc moins de réduire l'endémie que de chercher à la supprimer. On a l'impression d'être près du but à atteindre, c'est à dire l'extinction de la maladie.

La prophylaxie chimique peut être organisée, soit aveuglément, soit après avoir écarté par le dépistage les individus atteints de la maladie du sommeil, ce qui exige une prospection préalable approfondie, longue et coûteuse.

Mais même dans cette seconde éventualité, nous avons à tenir compte des trois faits suivants:

1. Un certain nombre d'individus échappe fatalement aux opérations de prophylaxie: leur pourcentage varie avec la psychologie locale des indigènes, leur mode de vie, l'autorité des chefs, etc.

2. Un certain nombre d'individus qui ont reçu correctement les doses habituelles de diamidine pourrait ne pas être protégé et rester par conséquent vulnérable à la piqûre infectante. Il ne nous est pas possible actuellement d'évaluer l'importance de ces cas. À première vue, elle paraît faible. En revanche, pour ce qui est de la durée de la protection conférée par les injections, on est loin d'être d'accord. Il s'agit pourtant d'une donnée de la plus haute importance, puisqu'elle doit commander le rythme des campagnes successives. Le but final étant d'arrêter le cycle biologique du virus, il y aurait lieu, nous semble-t-il, de ne pas dépasser 4 mois entre la 1ère et la 2ème cam-

[1] La dose administrée doit être de 4 mg base/kg.

[2] Nous rappelons que le MSbB (Friedheim) a donné, aux essais, des résultats très prometteurs et mérite d'être retenu pour des études ultérieures.

pagne, les suivantes pouvant s'espacer 2 fois de 6 en 6 mois. Si la trypanosomiase se comporte comme les autres maladies infectieuses et parasitaires, le fait que les indices descendent en dessous d'un certain chiffre (qu'il faudra rechercher à l'avenir) signifie que la population intéressée n'est plus menacée immédiatement et que l'on peut arrêter les campagnes de prophylaxie. Dans cette éventualité favorable, des mesures permanentes de surveillance doivent néanmoins être maintenues de façon à pouvoir intervenir en temps utile, en cas d'alerte.

3. Un troisième groupe, enfin, comprend les individus qui étaient déjà infectés au moment de l'administration du produit, mais chez lesquels les examens de dépistage sont restés négatifs. L'importance de ce groupe n'est pas négligeable si l'on prend comme base de comparaison les résultats que donne la prospection habituelle (examens divers pratiqués à un seul moment comme cela se fait dans les campagnes de prophylaxie chimique) et ceux que l'on obtient par des examens répétés plusieurs jours de suite. Pour ce qui nous concerne, nous serions tentés d'évaluer de 10 à 15 pour cent le nombre des cas qui échappent lors des opérations collectives de dépistage les mieux conduites. Or, l'introduction de la prophylaxie chimique a une incidence sur la clinique qui mérite d'être soulignée. En effet, les diamidines, même à petite dose, camouflent l'évolution de la maladie. Qu'il s'agisse d'individus qui étaient porteurs de trypanosomes non décelés au moment de l'injection prophylactique, ou d'individus qui se sont infectés par après et chez lesquels le mécanisme de protection n'a pas joué, le grand risque réside dans le fait que l'évolution se fera le plus souvent à bas bruit, les trypanosomes étant pratiquement impossibles à déceler, le diagnostic n'apparaîtra que sur les données biologiques du stade nerveux, ce qui ne manque pas de compliquer la thérapeutique et d'assombrir le pronostic. Ces considérations ne peuvent toutefois pas faire hésiter à entreprendre des campagnes de chimioprophylaxie dans les régions d'endémie *du moment qu'on est assuré de pouvoir les poursuivre jusqu'au bout,* puisqu'on sacrifie sciemment des individus devant le problème collectif à résoudre.

Nous n'aborderons pas ici l'éventualité du développement toujours possible d'une certaine chimio-résistance aux diamidines. Ce problème, qui est du plus haut intérêt d'un point de vue purement

scientifique, ne paraît pas à l'heure actuelle créer une menace réelle, pas plus que jadis avec le Bayer 205, contrairement à celui que soulève l'introduction de nouveaux arsenicaux ou organo-métalliques en général [1].

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[1] Le Comité Scientifique International de Recherches sur les Trypanosomiases avait inscrit la question de la chimioprophylaxie au programme de sa 7ème session (Bruxelles, 25 au 30 octobre 1958). Il a formulé les recommandations suivantes:

*a*) Le Comité *confirme* que la chimioprophylaxie par les diamidines a donné, partout où elle a été utilisée, d'excellents résultats et a permis de réduire considérablement l'incidence de la trypanosomiase due à *T. gambiense*.

*b*) Le Comité *recommande* l'emploi de cette méthode partout où elle est applicable et indiquée.

*c*) Le Comité *reconnaît* que les facteurs limitant l'emploi de cette méthode sont surtout d'ordre administratif, social et économique.

*d*) Le Comité *souhaite* que des recherches soient entreprises afin de préciser le mode d'action des produits employés en chimioprophylaxie.

*e*) Le Comité *souligne* la valeur de la méthode du dépistage-traitement, soit seule, soit associée à toute opération de chimioprophylaxie de masse.

*f*) Le Comité *considère* que, faute de données suffisantes, il n'est pas encore possible de définir avec précision une doctrine de valeur générale, quant à l'époque à laquelle on peut mettre un terme aux opérations de chimioprophylaxie ou les suspendre dans une zone donnée.

*g*) Le Comité *désire souligner* que le succès de toute campagne de lutte contre la trypanosomiase peut être gravement compromis par des conditions politiques défavorables. Ce succès est étroitement lié à la coopération active des populations locales et des autorités administratives.

# TRAITEMENT ET CHIMIOPROPHYLAXIE DES TRYPANOSOMOSES ANIMALES

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Ainsi que nous l'avons signalé dans un travail antérieur (1955), le problème de la lutte contre les trypanosomoses animales est extrêmement complexe en raison de leur vaste répartition géographique, de la multiplicité des espèces de flagellés, des divers modes de transmission naturelle, de la variété des espèces animales atteintes, de leur réceptivité différente à l'un ou l'autre trypanosome, de la réponse variable des divers trypanosomes aux médicaments ...

Dans la présente note, forcément limitée, nous insistons particulièrement sur les trypanocides expérimentés au cours des 15 dernières années car ils constituent un progrès considérable dans le traitement et la prophylaxie des trypanosomoses.

D'autre part, nous exposons essentiellement les travaux appliqués à l'Afrique tropicale parce qu'ils sont, et de beaucoup, les plus nombreux, du fait que ce continent est le plus infecté, que les glossines, vecteurs principaux des trypanosomes, forment une population dense d'insectes spécifiquement africains, que les espèces de trypanosomes y sont quasiment toutes représentées et qu'enfin les trypanosomes sont, pour l'Afrique, un fléau.

On remarquera que les recherches, en dehors du laboratoire, portent surtout sur les bovins, qui sont pour les autochtones la source principale de viande, lait, travail et conditionnent par là même l'évolution des populations.

Enfin, parmi les trypanosomes bovins, deux espèces servent habituellement aux investigations: *Trypanosoma congolense* et *Trypanosoma vivax*, le premier parce qu'il est plus «actif», le second parce qu'il est géographiquement le plus répandu.

Nous classons les trypanocides étudiés dans les rubriques ci-dessous: *composés uréiques, diamidines, dérivés de la phénanthridine, dérivé de la quinaldine, associations de trypanocides, complexes de suramine*. Nous terminons par un aperçu sur la chimiorésistance et des commentaires sur la chimiothérapie et la chimioprévention dans la pratique.

## COMPOSÉS URÉIQUES

Le seul composé uréique, et le plus ancien, qui ait encore quelques indications est le 205 Bayer ou 309 Fourneau, urée symétrique du métaminobenzoyl métamino-paraméthylbenzoyl-naphtylamino-4-6-8-trisulfonate de sodium.

*Propriétés curatives.* — Il est employé dans le traitement du surra *(Trypanosoma evansi)*, en Asie, chez le cheval, les bovins et les bubalins (Vittoz, 1955).

La dose préconisée est de 1 cg/kg chez le cheval, 2 à 3,5 cg/kg chez le bœuf.

Selon Velu et Zottner (1953), la dose de 1 cg/kg est trop élevée et toxique, non seulement pour le cheval, mais aussi pour les autres espèces animales. Elle peut entraîner des congestions rénales aiguës ou des néphrites chroniques (Zottner, 1955).

Chez les dromadaires du Sahara, fréquemment infectés par *T. berberum* Sergent, 1903, trypanosome du groupe *evansi* (qui provoque l'affection appelée *el debab* en Afrique du Nord), les observations les plus récentes sont celles de Chaumard (1955). Au cours de quatre années (1950-1954), il traite 3.614 animaux avec une dose individuelle uniforme de 5 g de moranyl. Il associe parfois ce médicament au trypoxyl (3 g moranyl + 3 g trypoxyl). Les réactions sont les suivantes: quelques abcès sans gravité, chez les femelles une baisse de la lactation pendant 3 ou 4 jours, quelques cas de cécité passagère. Il n'est pas signalé d'avortement chez les chamelles pleines. Sur les 3.614 sujets traités, 3.565 sont guéris, 49 meurent.

Le moranyl est encore couramment employé en A.O.F. dans le traitement du chien infecté par *T. brucei.*

*Propriétés préventives.* — Les propriétés préventives de ce produit, qui ouvrirent la voie à la prophylaxie chimique, ne sont guère utilisées actuellement que chez le dromadaire vivant dans les zones à *T. evansi.* Chaumard (1955) traite préventivement 337 animaux et 3 seulement sont atteints ultérieurement. La durée de la protection est d'environ un an.

La prophylaxie de la dourine *(T. equiperdum)* chez le cheval, à la dose de 3,5 mg/kg, à doses répétées (Velu et coll., 1938), est abandonnée. Même à doses faibles, le rein est cependant lésé, quoique discrétement (hypochlorurie précoce et albuminurie légère). L'inconvénient le plus grave reste l'installation d'une frigidité passagère fort gênante pour des étalons en pleine période de monte (Zottner, 1952).

## DIAMIDINES

Parmi les diamidines, un dérivé, le *bérénil,* diacéturate de di (4-amino phényl) triazène (N 1,3), découvert par Jensch (1955), a fait l'objet de recherches, encore peu nombreuses.

Fussganger (1955) indique les premiers résultats obtenus en laboratoire. Peu actif contre *T. brucei,* son pouvoir curatif est surtout net contre *T. congolense,* à la dose de 2,5 mg/kg chez la souris (par voie sous-cutanée) et chez le chien (par voie intramusculaire). Davey (1957) ne réussit à stériliser la souris infectée par *T. congolense* (souche Ouganda) qu'avec une dose de 5 mg/kg.

La *toxicité* de ce produit est faible par voies sous-cutanée et intramusculaire, plus accusée par voie intraveineuse. Sa concentration dans le sang est maximum entre 3 et 5 heures après l'injection, et son élimination par voie rénale est quasi totale après 16 heures.

Finelle (1957), dans la région de l'Oubangui-Chari (A.E.F.), note, chez les bovins, qu'à la dose de 3,5 mg/kg (par voie sous-cutanée ou intramusculaire) aucune réaction locale ni générale n'est enregistrée, les femelles gravides, les jeunes, les animaux en mauvais état général tolèrent bien le traitement. Les phénomènes d'intoxication (signes nerveux graves) n'apparaissent qu'à partir de 14 mg/kg. En

adoptant la voie intraveineuse, les signes nerveux interviennent de façon précoce (quelques minutes) à des doses de 7 à 10,5 mg/kg.

*Propriétés curatives.* — Bauer (1955), au Mozambique effectue les premiers traitements sur des bovins naturellement infestés par *T. vivax* et (ou) *T. congolense* et *T. brucei*. A la dose de 2 ou 3 mg/kg par voie intramusculaire, la plupart des trypanosomes disparaissent de la circulation sanguine périphérique en 24-48 heures. Une dose de 1 mg/kg suffirait pour éliminer *T. congolense*.

Milne et coll. (1955), au Tanganyika, confirment ces données en ce qui concerne *T. congolense* (le critère de guérison est l'absence de trypanosomes dans le sang pendant 150 jours). La dose utilisée est 2 mg/kg. Au Kenya (1956), à la dose de 3 à 5 mg/kg, en sous-cutanée, on obtient l'élimination de *T. congolense* et *T. vivax*, résistants à la fois à l'antrycide et aux composés de la phénanthridine.

Finelle (1957), opérant dans des zones à glossines et sans glossines, sur des bovins infectés par *T. vivax* et *T. congolense*, obtient la disparition des hémoparasites pendant des périodes de 2 à 4 mois avec des doses de 1,5 à 3 mg/kg. Sur les malades dont l'affection est ancienne le traitement échoue.

Les souches de trypanosomes résistantes à l'antrycide sont sensibles au bérénil.

*Propriétés préventives.* — D'après Milne et coll. (1955), des bovins guéris, réinfectés au bout de 150 jours, ne sont pas protégés. Finelle (1957) aurait eu des résultats favorables sur des bovins exportés par voie de terre, traversant des zones à glossines, et traités respectivement avec l'antrycide pro-salt, le chlorure de dimidium et le bérénil.

Des expériences plus nombreuses et plus complètes sont à envisager.

## DÉRIVÉS DE LA PHÉNANTHRIDINE

### I — Bromure (ou chlorure) de dimidium.

Morgan et Walls (1938) font connaître le premier dérivé de la phénanthridine le S 937 expérimenté chez la souris par Browning et coll. (1938). Ce corps est le chlorure de 7-amino-9 (*p*-aminophényl) 10 méthyl phénanthridinium.

Il présente un pouvoir trypanocide marqué contre *Trypanosoma congolense*, démontré sur les bovins, en Afrique, par Hornby et coll. (1943).

Il est rapidement abandonné à cause de sa faible solubilité, de l'obligation d'utiliser la voie intraveineuse pour l'administration et de répéter les injections pour assurer la guérison.

Le S. 1553, qui pallie partiellement ces défauts, est le bromure de diamino-2-7-phényl-9-méthyl-10-phénanthridinium que Launoy et coll. (1947) appellent *bromure de dimidium*. La solubilité de ce produit étant encore insuffisante, certaines firmes commercialisent le *chlorure de dimidium*, dont les propriétés sont identiques, mais la solubilité supérieure, grâce à l'acide salifiant la base phénanthridine.

*Propriétés curatives.* — Les premiers essais sont effectués par Carmichael et Bell (1944), en Ouganda, et se révèlent satisfaisants contre la trypanosomiase bovine à *T. congolense*.

De très nombreux travaux et une large vulgarisation confirment sa valeur dans la plus grande partie de l'Afrique: Stewart (1946) en Gold Coast, Van der Elst (1948), Guyaux (1948) au Congo Belge, Receveur (1950) au Tchad ...

Son action est également favorable contre la trypanosomiase des bovins et des chevaux à *T. vivax*, d'après Stewart (1946) en Gold Coast, Fiennes (1946) dans l'Est africain, Blommaert (1948) au Congo Belge, Mornet et Mahou (1949) en A.O.F., Receveur (1950) au Tchad ... Cependant, la cure est plus aléatoire avec ce trypanosome, chez les bovins en particulier, car il provoque parfois une infection chronique ou latente. Il est admis qu'en dehors de ces deux trypanosomes, les autres, tels *T. brucei* (bovins), *T. evansi* (chevaux et chameaux), *T. simiae* (porcs), sont irrégulièrement sensibles.

La posologie à adopter varie suivant les auteurs: 0,8 à 2 mg/kg. Actuellement, on admet généralement 1 mg/kg.

La voie d'introduction est d'abord la sous-cutanée, qui ne causerait, selon les promoteurs, qu'une faible irritation locale. En fait, les réactions sont souvent importantes (Barnett, 1946; Randall et Beveridge, 1946; Evans, 1948; Mornet et coll., 1949), se manifestant par un œdème chaud, douloureux, crépitant, allant jusqu'à la formation d'abcès nécrotique, ou d'escharre.

La voie intramusculaire entraîne aussi des nécroses profondes

avec boiterie, extrêmement graves pour des animaux transhumants ou exportés sur pied.

Ces inconvénients nous font personnellement adopter, dès 1950, la voie intraveineuse, mais là encore les risques de périphlébite chez des animaux habituellement indociles sont sérieux. Et ce procédé est peu pratique pour de grands effectifs bovins, d'élevage extensif, communément rencontrés en Afrique tropicale, l'opération devant être effectuée le plus souvent sur l'animal couché.

Au Kenya, actuellement, il est prescrit d'administrer le bromure de dimidium (comme les autres trypanocides) dans le fanon, ce qui évite, lors d'abattage pour la boucherie, que la viande et la peau soient endommagées. En effet, au niveau de ce point d'inoculation, la réaction inflammatoire reste limitée, par suite de la densité des tissus.

*Propriétés préventives.* — Elles sont indéniables envers *T. congolense* et *T. vivax*, mais la durée de la prévention est courte, un mois environ (Crawshaw, 1947; Deom, 1950).

Au cours d'essais pratiqués sur des animaux exportés sur pied de Haute Volta en Gold Coast, à travers des zones à tsé-tsés, Mornet et coll. (1951 et 1952) obtiennent la protection d'un tiers des animaux pendant 8 semaines.

*Toxicité.* — L'enthousiasme des débuts en faveur de ce trypanocide, dont les qualités sont indéniables, baisse assez rapidement à la suite de la fréquence des accidents toxiques généraux observés.

Ils sont d'autant plus frappants qu'ils se manifestent «à retardement». Signalés d'abord par Randall et Beveridge (1946), ils n'apparaissent pas chez tous les animaux et il n'est pas aisé d'expliquer la sensibilité de certains sujets.

De 1946 à 1954, de nombreux observateurs relatent ces accidents dans toute l'Afrique, particulièrement dans l'Est africain et le Congo Belge. Ils sont rapprochés de ceux provoqués par la photosensibilisation, mais si la symptomatologie présente certaines analogies avec ce syndrome, l'influence solaire n'est pas toujours déterminante, car ils s'observent sur des sujets maintenus à l'ombre.

Les manifestations interviennent de la 5$^{e}$ à la 7$^{e}$ semaine après l'injection.

Le tableau symptomatologique est schématiquement le suivant: l'animal s'amaigrit, du larmoiement apparaît, quelquefois du jetage.

La peau, en certains endroits, devient hypersensible, œdématiée, érythémateuse. Tantôt les lésions sont discrètes (bases des cornes, pourtour du mufle), tantôt elles sont étendues, accompagnées ou non du prurit. La «photodermite» atteint en principe plus fréquemment les parties claires de la robe. Au début, les signes généraux sont plus ou moins nets: inappétence, chute de la sécrétion lactée, hyperexcitabilité.

Si la maladie évolue de façon défavorable, les lésions oculaires s'aggravent (kératite, ulcération de la cornée). La peau lésée présente des escharres, de la nécrose. L'appareil digestif est à son tour touché: après une courte période de constipation, la diarrhée s'installe. Les signes nerveux s'intensifient: il y a incoordination des mouvements, ataxie locomotrice.

Gretillat (1953) essaye de classer les accidents, suivant la «dominante» clinique et la fréquence, en:

| | | | |
|---|---|---|---|
| Cutanés : | 65 % | des bovins malades, | 2 % de pertes |
| Digestifs: | 20 % | des bovins malades, | 20 % de pertes |
| Nerveux: | 15 % | des bovins malades, | 100 % de pertes |

Thorold et Plowright (1952) donnent les chiffres suivants:

Au Kenya, sur 12.600 bovins ayant reçu 1 mg/kg de bromure de dimidium, on note 992 réactions faibles (7,8 %), 501 sévères (3,9 %) et 185 morts (1,4 %).

À l'autopsie, les lésions essentielles sont: la cachexie, l'ictère généralisé, l'hépatomégalie avec hyperhémie.

À l'examen histopathologique, on constate (Plowright, Burdin et Thorold, 1952): une dégénérescence graisseuse périportale, avec habituellement congestion sévère limitée aux espaces portes et parenchyme adjacent. Il y a accumulation de pigment brun-doré (bilirubine?) dans les espaces portes et les cellules de Küppfer.

Le dysfonctionnement hépatique est confirmé *in vivo* par les recherches biochimiques qui montrent un test de Van der Bergh positif. Le désordre organique se manifeste par la diminution de poids des animaux corrélativement avec une élévation du taux d'azote non protéique et de la phosphatase alcaline du sérum.

L'ictère est du type toxique plutôt que par rétention.

L'étiologie des accidents et leur mécanisme ne sont pas nettement élucidés.

Wilde (1949) avance l'hypothèse que les lésions hépatiques, entraînées par ce trypanocide, élèvent dans le sang le taux des substances photosensibilisantes (hémoporphyrines), à une époque de l'année où les herbages contiennent de la chlorophylle et d'autres substances photodynamiques en abondance.

Evans (1948) et Gretillat (1953) accusent au contraire les carences alimentaires (en protéines surtout), associées à une déficience hépatique.

Herin et Thienpont (1954) font intervenir la conjonction de divers facteurs: atteinte hémoparasitaire latente (piroplasmose, babesiellose, theileriose ...), fréquente chez les bovins de l'Afrique tropicale, carences alimentaires, hépato-intoxication par le trypanocide, aboutissant à une insuffisance fonctionnelle du foie, incapable d'assurer le métabolisme normal des porphyrines.

Burdin et Plowright (1952) font le rapprochement clinique avec la déficience en trois métabolistes essentiels: choline, acide nicotinique, riboflavine. Ils rappellent que le mode de dégradation et de détoxification *in vivo* du bromure de dimidium n'est pas connu.

Il ne semble pas y avoir en tout cas de relation entre le dosage ou la concentration de la solution du produit et l'apparition des signes d'intoxication. Par contre, l'administration répétée de bromure de dimidium déclenche plus facilement le phénomène.

Sur 26 zébus réinoculés avec 1 mg/kg, Plowright, Burdin et Thorold (1952) notent que 19 (soit 73 %) présentent des signes de photosensibilisation, dont 2 meurent (soit 7,7 %).

Cette toxicité du produit restreint son usage, d'autant que les traitements préconisés: mise au vert des animaux, alimentation qualitativement et quantitativement supérieure, mise en stabulation à l'ombre, donnent des résultats irréguliers.

II — Bromure (ou chlorure) d'éthidium.

Woolfe (1952) rappelle que si le bromure de dimidium est un trypanocide puissant contre les infections de laboratoire à *T. congolense* chez la souris, il est moins satisfaisant dans la lutte contre la trypanosomiase bovine, pour les raisons suivantes:

1° Le rapport entre la dose toxique et la dose active est très bas.

2° Le produit provoque parfois des lésions hépatiques, se traduisant par des phénomènes retardés de «photosensibilisation».

3° L'injection sous-cutanée ou intramusculaire entraîne des lésions locales nécrotiques.

Ces sérieux inconvénients conduisent à rechercher d'autres produits «apparentés».

En modifiant le groupement quaternaire méthyl du bromure de dimidium et en le remplaçant par des chaînes plus longues, éthyl, *n* propyl et allyl, on obtient des produits plus actifs, dont le *bromure d'éthidium* (Watrins et Woolfe, 1952). Le bromure d'éthidium (bromure de 2:7 diamino-9-phényl-10-éthyl-phénanthridinium) serait, chez la souris, beaucoup moins toxique que le bromure de dimidium et beaucoup plus actif contre *T. congolense, T. brucei* (et *T. gambiense* et *T. rhodesiense*).

*Propriétés curatives.* — Après les premiers essais de Wilde et Robson (1953), au Tanganyika, dans la trypanosomose à *T. congolense* du zébu, Wilson et Fairchlough (1953), au Kenya, confirment la valeur du bromure d'éthidium, sous dosage de 0,5 à 4 mg/kg.

De même, Ford, Wilmhurst et Karib (1953*a* et *b*), en République Soudanaise, obtiennent de bons résultats contre l'infection à *T. vivax* et *T. congolense* chez les bovins.

Unsworth (1954*a*), au Nigeria, dans les infections à *T. vivax* des bovins, reconnaît les qualités trypanocides de ce produit (critère de la guérison: absence de flagellés dans le sang périphérique pendant 6 mois). Mais les doses employées sont de 1 à 2 mg/kg. Celles de 0,25 à 0,50 mg/kg échouent.

Robson et Milne (1954), au Tanganyika, préconisent une dose standard de 0,25 g, sans tenir compte du poids de l'animal, contre *T. congolense,* au début de l'infection.

Le même traitement, retardé, donne aussi satisfaction (Milne et Robson, 1955).

Par contre, au Congo Belge, Thienpont et Herin (1955) observent 8 rechutes sur 47 bovins infectés par *T. vivax* et traités à la dose de 1 et 2 mg/kg en sous-cutanée; rechutes, soulignons-le, tardives (4e au 7e mois après l'infection).

Malgré le nombre relativement faible d'interventions avec le

bromure d'éthidium, il semble que la dose curative soit d'environ 1 mg/kg.

La voie d'administration recommandée au Kenya (1955) est l'injection dans le fanon, la sous-cutanée en un autre point, comme l'intramusculaire, étant irritante. Dans ce dernier cas, il est signalé, toujours au Kenya, que les muscles peuvent être intéressés sur une profondeur de 10 cm et une longueur de 14 cm.

L'avantage de ce trypanocide sur le bromure de dimidium est son innocuité; il ne produit pas de lésions hépatiques, même à des doses de 4 mg/kg (Burdin, 1953; Unsworth, 1954*b*).

*Propriétés préventives.* — Leach et coll. (1955) obtiennent une protection partielle contre l'infection à *T. congolense* pendant 4 semaines avec 1 mg/kg et pendant 6 semaines avec 5 mg/kg.

En République Soudanaise, en 1955, 100 zébus ayant reçu soit 1 mg/kg soit 5 mg/kg, à intervalles différents, sont infectés avec du sang citraté riche en *T. congolense* ou *T. vivax,* par voie intramusculaire (la voie sous-cutanée ayant été suivie de réactions locales allant parfois jusqu'à la nécrose). Il est noté: avec *T. congolense,* 1 mg/kg donne une protection de plus de 4 semaines, portée à plus de 6 semaines avec 5 mg/kg; avec *T. vivax,* la protection est de 6 semaines.

En résumé, les résultats obtenus ne sont guère supérieurs, du point de vue curatif ou préventif, à ceux enregistrés avec le bromure de dimidium. Le seul véritable avantage est son absence de toxicité, même à des doses élevées.

### III — Prothidium.

Trois composés, combinant la structure de l'antrycide et du dimidium (ou de l'éthidium), sont retenus par Watkins et Woolfe (1956) dans la prophylaxie de l'infection à *T. congolense* chez la souris.

Parmi ces trois corps, le RD 2801, expérimenté par Robson et Milne (1957), au Tanganyika, dans la trypanosomose à *T. congolense* des zébus, confère la plus longue durée de protection.

L'appellation commerciale de *prothidium* a été donnée à cette préparation de 10:1-diméthylbromure de 2-amino-7-(2-amino-6-méthyl-4-pyrimidylamino)-9-*p*-aminophénylphénanthridine.

Les propriétés préventives ont été particulièrement étudiées.

*Propriétés préventives.* — Les essais en laboratoire ayant montré l'activité particulière du prothidium comme agent prophylactique des trypanosomoses, l'expérimentation pratique sur le bétail s'est attachée essentiellement à la prévention des infections à *T. congolense*, *T. vivax* et parfois *T. brucei*.

Au Tanganyika, Robson et Milne (1957), à la dose de 1 mg/kg, obtiennent une protection moyenne de 6 mois; à la dose de 2 mg/kg, une protection quasi-totale de 6-7 mois (également avec 4 et 6 mg/kg).

Au Kenya (1956), les résultats sont excellents aux doses de 2 et 4 mg/kg: protection de 6 mois et plus de 6 mois contre *T. congolense*, *T. vivax* et *T. brucei*. Les zébus sont plus favorablement influencés que les bœufs sans bosse, les premiers ayant sans doute une résistance naturelle plus grande à la trypanosomose [1].

Dans la République Soudanaise, la durée de protection contre *T. congolense* varie de 6 à 8 mois, à la dose de 2 mg/kg.

Au Nigeria, elle est de 4 à 6 mois, à la dose de 2 et 5 mg/kg.

Au Tchad (Laboratoire de Farcha), Magimel [2] (1958, communication personnelle) souligne qu'on ne peut dépasser la dose de 5 mg/kg. Il estime que le déparasitage anthelminthique des bovins d'expérience confère à ces derniers une résistance accrue aux doses élevées de trypanocide.

Suivant une posologie de 2, 3, 4 et 5 mg/kg, la protection en laboratoire est de 4, 5 mois et plus contre *T. vivax* et *T. brucei*.

Par contre, pour des animaux placés dans une galerie forestière, à la dose de 2 mg/kg la protection ne dépasse pas 4 mois pour *T. vivax* et *T. congolense*.

Le médicament est injecté, par les divers auteurs, soit par voie intramusculaire, soit par voie sous-cutanée, la voie intraveineuse étant contre-indiquée.

Cependant, au Kenya (1956), la voie intramusculaire (cuisse) entraînerait, à la dose de 2 à 4 mg/kg, en solution à 2 %, les mêmes accidents que les autres composés de la phénanthridine: vastes lésions douloureuses, nécrotiques, disparaissant au bout de quelques semaines, mais entraînant une baisse de poids des animaux considérable. La

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[1] La sensibilité de ces bovins est inverse en A.O.F.

[2] Décédé en avril 1958.

voie sous-cutanée, dans le fanon, est recommandée. On peut, à cet endroit, injecter sans incident jusqu'à 10 mg/kg.

De façon générale, il est reconnu que la toxicité du prothidium est faible: pas de réaction retardée, pas de lésions hépatiques décelables, pas de modifications du taux d'hémoglobine, de la bilirubine du sérum ou de la phosphatase alcaline. Il a été signalé cependant au Nigeria et en République Soudanaise des accidents toxiques (Brownie et coll., 1957) jusqu'à présent inexpliqués.

## DÉRIVÉ DE LA QUINALDINE

### Antrycide.

L'*antrycide*, nom commercial des sels (chlorure et méthylsulfate) 1:diméthylés de la 4-amino-6 (2-amino-6' méthylpyrimidil-4'-amino) quinaldine, est présenté par Curd et Davey (1949) comme possédant une action trypanocide marquée, curative et préventive. Davey (1950*a*) fait le point des travaux expérimentaux, en laboratoire et sur le terrain. Le même auteur (1957) expose une synthèse des informations les plus récentes.

Dès 1949, il souligne, et c'est une des particularités de ce produit, que les propriétés des deux sels utilisés, méthysulfate et chlorure, sont très différentes.

Le méthylsulfate, très soluble dans l'eau (environ 33 %), est très rapidement absorbé (par voie sous-cutanée) et sa concentration dans le sang atteint, dans un bref délai, un taux élevé. Le chlorure, au contraire, peu soluble (0,12 %), se résorbe lentement et la concentration sanguine reste faible.

Il s'ensuit que si leur activité sur le trypanosome est superposable, leur emploi est différent par suite de leurs propriétés pharmacologiques particulières. Le méthylsulfate, dont l'absorption est accélérée, est surtout curatif, alors que le chlorure, dont la résorption est lente par suite du «dépôt» qu'il constitue sous la peau (ou dans le muscle), est essentiellement préventif.

Par ailleurs, l'intérêt de l'antrycide tient à sa polyvalence plus marquée que celle des autres produits envers les différentes espèces de trypanosomes des animaux domestiques.

Si l'on prend comme référence la classification de Hoare (1957) pour les trypanosomes d'intérêt vétérinaire et médical, son action s'exerce:

Groupe *vivax* — Sur *T. vivax*
Groupe *congolense* — Sur *T. congolense* et, à un degré moindre, sur *T. simiae.*
Groupe *brucei* — Sur *T. brucei, T. evansi, T. equinum, T. equiperdum.*

Il est évident que cette activité a des degrés suivant les espèces de trypanosomes, et certains essais ont été trop limités pour être concluants. Il est admis cependant que le caractère trypanocide de l'antrycide s'affirme plus nettement envers *T. congolense, T. vivax* et *T. equiperdum.*

*Propriétés curatives du méthylsulfate d'antrycide.* — Les essais de Davey (1950*b*) portent au début sur les infections à *T. congolense* chez les bovins, en République Soudanaise et dans l'Est africain. La dose unique de 1 à 2 mg/kg est injectée.

Ces résultats sont confirmés par de nombreux chercheurs: Thienpont (1950), Wery (1950), Schoenaers (1950), Tobback (1950), au Congo Belge, Cruz Ferreira et Tendeiro au Mozambique (chez les bovins).

La dose pratique est devenue 5 mg/kg. Des traitements en masse sont pratiqués en République Soudanaise par Evans (1950) sur plusieurs centaines de milliers de bovins, et en Ouganda, de 1950 à 1955. La conclusion des Services vétérinaires de ce territoire est que si l'antrycide ne constitue pas une solution définitive à l'éradication, il est cependant un excellent trypanocide. La politique de traitement en masse a modifié la situation sanitaire, de sorte que la trypanosomose n'est plus enzootique mais n'évolue qu'en foyers limités.

Au Kenya, au Nigeria, en A.O.F., les rapports consultés sont, dans l'ensemble, favorables.

Les auteurs précités reconnaissent que *T. vivax* est également sensible à l'antrycide, tout en étant moins rapidement éliminé de la circulation périphérique que *T. congolense.* Les rechutes enregistrées tiennent souvent à la forme latente ou chronique de l'affection provoquée par le premier.

Les renseignements concernant l'action de l'antrycide sur *T. brucei* sont moins nombreux mais généralement favorables. Les résultats du traitement de la trypanosomose du porc à *T. simiae* sont discordants, favorables pour Wilson (1949) en Ouganda, médiocres pour Unsworth (1952) au Nigeria.

Si, pour *T. evansi,* il existe des opinions divergentes, elles sont moins tranchées. En République Soudanaise les résultats sont satisfaisants dans la trypanosomose du chameau (dose: 5 mg/kg), de même en Somalie Italienne (Pelligrini et Bonelli, 1951) et en Iran (Rafyi et Maghami, 1953). Au Soudan Français (1955), certains incidents: abcès profonds, guérisons incomplètes, ont amené à reprendre l'ancien traitement au moranyl.

En Inde, chez les chevaux et les bovins, la guérison est obtenue avec 5 mg/kg pour les premiers et 3 mg/kg pour les seconds (Ray et coll., 1953). En Indochine, Chary et Bocquet (1955) n'obtiennent chez le cheval que des résultats partiels, mais excellents chez le chien (plusieurs injections).

Dans le mal de Cadéras expérimental *(T. equinum),* Nery et coll. (1953) signalent quelques succès chez le cheval (5 mg/kg méthylsulfate + chlorure), également chez le chien, mais avec quelques rechutes.

En ce qui concerne *T. equiperdum,* les essais de Zottner (1952) sont concluants. Chez les chevaux, la dourine est curable à la dose de 5 mg/kg.

*Propriétés préventives du pro-salt d'antrycide.* — Dans la pratique, la prévention est obtenue par un mélange de méthylsulfate (3 parties) et chlorure (4 parties), connu sous le nom de *pro-salt d'antrycide.*

Après que Davey (1950a) eut montré qu'une seule dose permet de protéger les bovins pendant plusieurs mois contre les infections à *T. congolense* et *T. vivax,* des essais sont effectués dans divers territoires, en particulier par Unsworth et Chandler (1952), Fiennes (1952), etc.

Dans la pratique, on cherche à maintenir les troupeaux bovins dans les zones à tsé-tsés; et si la protection n'est pas aussi durable qu'on le pensait au début (4 à 6 mois), des injections répétées tous les deux mois permettent aux animaux de prospérer en Rhodésie du nord, au Tanganyika, au Kenya, en Ouganda. Dans ce dernier ter-

ritoire, on estime que les réinstallations dans les régions abandonnées par les colons sont possibles grâce à ce procédé.

Mornet et coll. (1951 et 1952) entreprennent plusieurs expériences pratiques de protection des bovins d'exportation se rendant à pied des zones d'élevage septentrionales de l'A.O.F. vers les marchés du sud. La valeur comparée de divers trypanocides est nettement en faveur de l'antrycide. Unsworth et Birkett (1952), au Nigeria, arrivent aux mêmes conclusions.

Récemment Soltys (1955) étudie la résistance à *T. congolense* acquise par le bétail zébu traité prophylactiquement par l'antrycide pro-salt dans une région d'enzootie de l'Est africain. Ces recherches avaient pour but de vérifier si des animaux soumis pendant longtemps (28 mois) à des injections bimensuelles d'antrycide pouvaient résister à l'infection par *T. congolense* après une interruption prolongée du traitement. Les résultats sont concluants et, s'ils sont confirmés, une nouvelle méthode de lutte contre les trypanosomoses pourrait être envisagée.

À ce propos, signalons la tentative de Roubaud (1952) d'assurer la prophylaxie des infections trypanosomiennes (*T. congolense* et *T. vivax*) par l'action de l'antrycide s'exerçant directement dans l'organisme des glossines sur les flagellés qu'elles convoient. Elle échoue, comme elle a échoué avec les autres trypanocides.

Contre la dourine (*T. equiperdum*), les essais de Zottner (1952) sont intéressants, la protection des chevaux étant de trois mois, période suffisante puisqu'elle correspond à la période de monte.

La protection contre *T. evansi* serait constante selon les uns, irrégulière selon les autres. Des recherches sont en cours en A.O.F. en ce qui concerne l'infection des chameaux.

*Toxicité.* — Que ce soit à titre curatif ou préventif, la voie sous-cutanée et la voie intramusculaire sont employées indifféremment pour l'administration du produit, mais les réactions locales sont fréquentes et si importantes parfois qu'au Kenya on tend à injecter la solution au niveau du fanon. Les autres lieux d'injection entraînent trop souvent des dégâts importants du muscle et de la peau. La voie intraveineuse est contre-indiquée.

La toxicité générale de l'antrycide est dûe surtout au méthylsulfate, par suite de la rapidité de son absorption.

Le cheval est l'animal le plus sensible; aussi de grandes précautions doivent être prises à son égard. Vaysse et Zottner (1950) signalent des phénomènes impressionnants dans les quelques minutes suivant l'injection: inquiétude, dyspnée, sudation profuse, coliques, qui pourraient relever d'une congestion générale du système capillaire par troubles vago-sympathiques.

Chez les bovins, les accidents, sans être aussi fréquents que chez les chevaux, ne sont pas exceptionnels: ptyalisme, incoordination des mouvements, accélération du rythme respiratoire et cardiaque, parfois chute sur le sol.

Les troubles sont généralement passagers mais peuvent entraîner la mort. À l'autopsie, on note une gastro-entérite hémorragique, de l'œdème pulmonaire, une abondante sérosité dans la cavité thoracique et sous-péricardique, des lésions rénales.

Les accidents sont évidemment plus fréquents avec les sur-dosages, mais sont signalés avec la dose normale, 5 mg/kg.

Il faut souligner que l'état d'entretien des animaux, leur âge, le degré d'infestation par les parasites intestinaux ont un rôle adjuvant dans l'apparition des signes toxiques.

Nous n'avons pas encore étudié le phénomène de *chimiorésistance* qui a été soulevé plus particulièrement à propos de l'antrycide, mais qui intervient avec la plupart des trypanocides. Nous le traiterons dans un chapitre séparé.

## ASSOCIATIONS DE TRYPANOCIDES

La thérapie synergique trypanocide, proposée par Launoy dès 1931, connut de nombreuses applications et reste toujours une méthode précieuse.

Dans la pratique et sur les gros effectifs bovins, elle est d'une application plus malaisée que l'administration d'un seul trypanocide.

Récemment elle a été encore utilisée par Chaumard (1955) contre *T. evansi* du dromadaire en cumulant l'action du moranyl et du trypoxyl.

Finelle (1952), en laboratoire, associe avec succès la lomidine au chlorure d'antrycide (1 mg/kg de chaque produit) contre *T. congolense* ou *T. brucei* chez la souris.

## COMPLEXES DE SURAMINE

Williamson et Desowitz (1956) décrivent brièvement une méthode destinée à étendre dans le temps l'activité prophylactique des trypanocides actuels par combinaison avec la suramine (= naganol = moranyl = antrypol ...), sous forme de complexes, et les résultats préliminaires obtenus sur le bétail.

Williamson (1957) expose en détail l'activité prophylactique des complexes de suramine contre *T. congolense* chez les petits animaux de laboratoire, et fait un bref historique de cette question.

La première observation d'un complexe, suramine-pentamidine, semble devoir être attribuée à Guimarães et Lourie (1951)[1], qui montrent que la suramine peut inhiber de nombreux effets toxiques et pharmacologiques de la pentamidine, par la formation d'un sel complexe précipitable. Par contre, ils agissent de concert (sinon en synergie) comme agents chimiothérapiques.

Beaudiment et Zozo (1951), puis Beaudiment, Cauvin et Leproux (1954) expérimentent chez l'homme un moranylate de lomidine (4891 R.P.).

En laboratoire, César et coll. (1954), Schneider et Montezin (1954) montrent la valeur de ce complexe chez le rat infecté avec *T. brucei* et apportent la preuve de son action synergique.

Williamson (1957) indique ensuite les complexes expérimentés: suramine + bromure de dimidium ou bromure d'éthidium ou antrycide ou cinnoline 528 ou bérénil ..., les relations qui régissent la formation de ces complexes, leurs qualités physiques et chimiques. Il conclut que l'activité des complexes est due en particulier à leur forme de «dépôt» prophylactique (comme celui provoqué par le chlorure d'antrycide).

Desowitz (1957) décrit les résultats obtenus chez des bovins au cours d'une série d'expériences sur la durée de protection conférée par un certain nombre de complexes de suramine, avec le diméthyl-

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[1] Toute la bibliographie sur l'historique des complexes suramine se trouve dans l'article de WILLIAMSON, J. — *Ann. Trop. Med. Parasito.*, **51**: 440, 1957. — Nous y renvoyons le lecteur.

sulfate d'antrycide, le bromure d'éthidium, le bérénil, le prothidium, le RD 2902 [1].

Malgré le petit nombre d'animaux d'expérience, il apparaît que le complexe à base d'éthidium est le plus prometteur, aux doses de 5 et 10 mg/kg.

La protection minimum conférée est de 216 et 385 jours. Des réactions locales relativement sévères sont observées à la suite de l'injection de ce produit.

Alors que le prothidium seul, à la dose de 2 mg/kg, confère une protection minimum de 144 jours, sous forme de complexe suramine, la dose peut être augmentée (sans danger) jusqu'à 10 mg/kg et protéger pendant 285 jours.

Le complexe avec le diméthylsulfate d'antrycide ne donne une protection de 132 et 167 jours qu'avec des doses élevées, respectivement 20 et 40 mg/kg.

Les complexes sont, à des degrés divers, moins toxiques que les trypanocides seuls, ceci étant surtout marqué pour le diméthylsulfate d'antrycide et le bromure d'éthidium.

En A.E.F., Magimel (1958), au Laboratoire de Farcha (Tchad), utilise deux complexes: le moranylate de lomidine et le moranylate d'éthidium.

Le moranylate de lomidine ne donne qu'une protection négligeable, en moyenne 27 jours.

Le moranylate d'éthidium, au contraire, confère une protection totale pendant plus de 210 jours, aux doses de 5, 10, 15 mg/kg par voie intramusculaire.

Les réactions locales sont parfois intenses, mais transitoires, disparaissant au bout de deux mois.

## CHIMIORÉSISTANCE

La chimiorésistance est un phénomène connu non seulement pour les trypanosomes, mais pour la plupart des microorganismes. Schnitzer et Grunberg (1957) développent longuement ce problème.

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[1] Un des trois composés qui combinent en partie la structure du dimidium et de l'antrycide, expérimentés par Robson, J., et Milne, A. H. — *Vet. Rec.*, **69**: 564, 1957.

Si, pour les trypanosomes, l'arsénorésistance est bien établie, elle ne constitue pas une particularité de ce produit, la plupart des autres trypanocides pouvant, sous certaines conditions, développer chez les flagellés des réactions semblables. La chimiorésistance peut être naturelle, assez rarement, acquise plus souvent, ou provoquée expérimentalement suivant un protocole relativement simple.

Parmi les trypanocides que nous venons d'étudier, aucun n'échappe à cette défaillance thérapeutique, sauf le bérénil. Et on accuse généralement (Wilson, 1953) l'emploi répété de médicaments trypanocides (antrycide, bromure de dimidium, bromure d'éthidium) dans les régions infestées de tsé-tsés.

Ainsi que le font remarquer Karib et coll. (1954), lors de traitements en masse il existe des occasions fréquentes de développer la résistance au médicament, telles que le sous-dosage à la suite d'estimations erronées du poids de l'animal, d'accidents de l'injection, de préparations imparfaites des solutions ou de réinfections au moment où le taux du médicament s'abaisse dans le sang.

C'est d'ailleurs ce que reconnaît Davey (1950) à propos de l'antrycide. Encore faut-il faire la différence entre le méthylsulfate et le chlorure. Le premier ne crée pas plus de souches résistantes que les autres trypanocides. Plus sérieux, écrit-il, est le danger de créer des trypanosomes résistants par l'emploi prophylactique de l'antrycide. En effet, au moment où la concentration décroissante de ce produit est tombée au-dessous du niveau nécessaire pour une protection complète, les trypanosomes injectés (ou inoculés) peuvent devenir résistants. Mais ce défaut de l'antrycide est inhérent à toute substance prophylactique.

Le danger est d'ailleurs beaucoup moins grand que ne le supposaient certains auteurs au début, en particulier Fiennes (1953). Unsworth (1954) montre que sur les rats, si on peut aisément rendre une souche de *T. congolense* résistante à l'antrycide, cette même souche, après plusieurs passages, sans traitement, perd sa résistance. Ormerod (1952), cité par Unsworth (1954), signale d'ailleurs la perte complète de résistance d'une souche de *T. equiperdum* chez la souris après «stockage», sans traitement pendant un mois, chez le cobaye.

Les craintes de Fiennes (1952) que l'abus des trypanocides, tel que l'antrycide prophylactique, entraîne de graves inconvénients, en

particulier celui d'entretenir des formes latentes de trypanosomoses susceptibles de favoriser la contagion, paraissent actuellement excessives.

Et d'ailleurs les travaux de Soltys (1955) sur la répétition des traitements par l'antrycide et leur intérêt pour accroître les défenses de l'organisme aux réinfestations semblent rassurants.

En ce qui concerne le bérénil, pour lequel on ne cite pas d'observations de chimiorésistance, nous pensons que cela tient plus au fait qu'il n'est pas utilisé comme prophylactique et que le nombre de traitements avec ce produit est encore limité. En effet, les diamidines, groupe auquel appartient le bérénil, développent aussi la chimiorésistance. Richet (1954), à propos de la lutte contre la trypanosomose humaine en A.E.F. et de la lomidinisation semestrielle, écrit:

> «La proportion des «suspects cliniques» semble donc croître avec le maintien et la répétition de la chimioprophylaxie semestrielle ...
>
> Si l'endémie cède de plus en plus de terrain, elle se «durcit»...
>
> Il semble que le trypanosome, vulnérable dans la circulation périphérique à la lomidine préventive, brûle les étapes et passe très vite dans le liquide céphalorachidien.»

On connaît les procédés proposés pour réduire la chimiorésistance: augmentation de la dose, emploi d'un autre trypanocide, utilisation des trypanocides associés, et peut-être des complexes trypanocides, etc.

Unsworth (1954), à propos de l'antrycide, recommande que les traitements ne soient pas, de toute façon, retardés jusqu'à la réapparition des parasites dans le sang.

Par ailleurs, l'augmentation de la dose de médicament ou l'injection d'une dose forte d'emblée n'est pas toujours possible, ainsi que le note Davey (1950), lorsque, pour le bromure de dimidium par exemple, et dans certaines infections à *T. congolense*, la dose active, quoique faible, est très proche de la dose toxique.

## COMMENTAIRES

Les recherches de ces dernières années ont accru de façon considérable l'arsenal thérapeutique destiné à la lutte contre les trypanosomoses.

Il faut retenir particulièrement parmi ces trypanocides: l'antrycide, le prothidium, les complexes suramine et, sous réserve d'essais plus étendus, le bérénil.

La polyvalence de la plupart d'entre eux permet d'envisager de combattre efficacement les infections mixtes si fréquentes en Afrique tropicale. C'est d'ailleurs contre les deux espèces de trypanosomes, *T. congolense* et *T. vivax,* qui tiennent une place si importante dans la pathologie africaine du bétail, que les efforts ont été dirigés.

Mais nul ne met en doute que c'est sur la prophylaxie, et la prophylaxie de masse, que repose, en grande partie, l'espoir de voir reculer les trypanosomes.

Dans ce domaine, l'intervention des complexes de suramine, si les succès récents sont confirmés, peuvent constituer un facteur décisif. Agissant à la fois en synergie (méthode Launoy) et sous forme de «dépôts» prophylactiques (comme le pro-salt d'antrycide), ils ont l'avantage d'autoriser l'emploi de doses fortes, en multipliant l'activité des produits tout en diminuant leur toxicité.

Par ailleurs les observations récentes sur la chimioprévention répétée au pro-salt d'antrycide, sur une longue durée et à intervalles courts, et la résistance ultérieure acquise, aux réinfestations, après interruption des injections, ouvrent des horizons nouveaux.

Mais qu'il s'agisse de traitement ou de prophylaxie, des plans communs pour les études devraient être envisagés, de façon à pouvoir tirer des conclusions valables des divers essais. Les protocoles sont en effet très variables suivant les chercheurs: tantôt les infections sont pratiquées «à la seringue», par des «glossines» en laboratoire, par l'exposition dans les galeries forestières, tantôt les examens de sang sont hebdomadaires, quotidiens, biquotidiens, tantôt les contrôles biochimiques sanguins sont complets, partiels ou nuls (test pour la toxicité du produit), tantôt les recherches de parasites dans le sang pour apprécier la guérison sont faites tous les deux mois, trois mois, quatre mois, six mois ...

En ce qui concerne la prophylaxie pratique, des principes ont été développées au Kenya (1956). Avant toute intervention, une enquête initiale est instituée pour la zone intéressée et détermine: les ressources en eau, en pacages; le nombre d'animaux à l'hectare, leur poids moyen; le comportement biologique des tsé-tsés; les espèces de trypanosomes et leur virulence, etc.

Ensuite, suivant la densité contrôlée des glossines, on combine: le choix du produit préventif, l'introduction d'un «stoppeur» (trypanocide destiné à combattre la chimiorésistance provoquée par un autre trypanocide), la production de l'immunité (répétition des injections, leur espacement suivant le produit, la densité des glossines ...).

On essaie ainsi de concilier l'efficacité et l'économie des interventions.

Il n'est, en conclusion, par douteux que l'orientation actuelle donnée aux recherches et aux méthodes de lutte contre les trypanosomoses et les résultats obtenus constituent des éléments très encourageants pour l'avenir.

## BIBLIOGRAPHIE

### 1° ARTICLES ET OUVRAGES D'ENSEMBLE

CEBE, J. — «Chimiothérapie des trypanosomoses animales», *Bull. Serv. Elev. A.O.F.*, **4**: 69, 1951.

CEBE, J. — *Traitement et chimioprophylaxie des trypanosomiases,* Journées Panafricaines de Zootechnie, Alger 17 au 23 octobre, **2**: 68, 1954.

DAVEY, D. C. — «The chemiotherapy of animal trypanosomiasis with particular reference to the trypanosomal diseases of domestic animals in Africa, *Vet. Rev. Annot.*, **3**: 15, 1957.

FINELLE, P. — *Traitement et chimioprévention des trypanosomoses animales.* Thèse Doctorat Vétérinaire, Alfort, 1952.

LAUNOY, L. — *Recherches sur les trypanosomoses expérimentales,* Index bibliographique. Hypothèses de travail. Declume Imprim. Lons-le-Saumier, 1949.

MORNET, P. — «Prophylaxie médicale des trypanosomiases animales», *Bull. Off. Int. Epiz.*, **44**: 68, 1955.

### 2° COMPOSÉS URÉIQUES

CHAUMARD, R. — *Rev. Corps Vet. Armée,* **1**: 10, 1955.

VELU, H., et ZOTTNER, G. — *Arch. Inst. Pasteur Alger,* **13**: 339, 1935.

VELU, H., ZOTTNER, G., et BELLE, G. — *Arch. Inst. Pasteur Alger,* **16**: 389, 1938.

Vittoz, R. — *Bull. Off. Int. Epiz.*, **44**: 83, 1935.
Zottner, G. — Communication personnelle, 1952.

### 3° DIAMIDINES

Bauer, F. — *Zeitschr. Tropenned. U. Parasit.*, **6**: 129, 1955.
Davey, D. G. — *Vet. Rev. and Annot.*, **3**: 15, 1957.
Finelle, P. — *Rev. Elev. Med. Vet. Pays. Trop.*, **10**: 231, 1957.
Fussganger, R. — *Vet. Med. Nachr.*, **3**: 146, 1955.
Jensch, H. — *Arzneim Forsch.*, **3**: 146, 1955.
Milne, A. H., Robson, J., et Lwebandiza, T. — *Vet. Rec.*, **67**: 280, 1955.
*Rapport Annuel des Services Vétérinaires du Kenya pour 1956.*

### 4° DÉRIVÉS DE LA PHÉNANTHRIDINE

#### A — BROMURE DE DIMIDIUM

Barnett, S. F. — *Vet. Rec.*, **58**: 325, 1946.
Blommaert, M. — *Bull. agr. Congo belge*, **39**: 663, 1948.
Browning, C. H., Morgan, G. T., Robb, J. V. M., et Walls, L. P. — *J. Path. Bact.*, **46**: 203, 1938.
Burdin, M. L., et Plowright, W. — *J. Comp. Path. Therap.*, **62**: 178, 1952.
Carmichael, J., et Bell, F. R. — *Vet. Rec.*, **56**: 495, 1944.
Crawshaw, H. A. — *J. Comp. Path. Therap.*, **57**: 13, 1947.
Deom, J. — *Ann. Soc. Belge Med. Trop.*, **30**: 25, 1950.
Evans, J. T. R. — *Vet. Rec.*, **60**: 418, 1948.
Fiennes, R. N. T. W. — *Vet. Rec.*, **58**: 606, 1946.
Gretillat, E. H. — *Bull. agr. Congo belge*, **44**: 797, 1953.
Guyaux, R. — *Ann. Soc. Belge Med. Trop.*, **28**: 179, 1948.
Herin, V., et Thienpont, D. — Bureau Perm. Interafr. Tsé-tsé et Trypano., 204/0, 1954.
Hornby, H. E., Evans, J. A., et Wilde, J. K. H. — *J. Comp. Path. Therap.*, **53**: 269, 1943.
Launoy, P., et Melle Prieur, M. — *Ann. Inst. Pasteur*, **73**: 58, 1947.
Mornet, P., et Mahou, R. — *Bull. Soc. Path.*, **42**: 355, 1949.
Plowright, W. — *J. Comp. Path. Therap.*, **62**: 136, 1952.
Randall, J. B., et Beveridge, C. G. L. — *Vet. Rec.*, **58**: 398, 1946.
*Rapport Annuel de la Section de Zoologie et de la Tsé-tsé* — Service Vétérinaire du Kenya — 1955.
Receveur, P. — *Bull. Off. Int. Epiz.*, **34**: 405, 1950.
Stewart, J. L. — *Vet. Rec.*, **58**: 133, 1946.
Thorold, P. W., et Plowright, W. — *J. Comp. Path. Therap.*, **62**: 136, 1952.
Van der Elst, O. — *Bull. Agr. Congo belge*, **39**: 131, 1948.
Wilde, J. K. H. — *J. Comp. Path. Therap.*, **59**: 48, 1949.

B — BROMURE D'ÉTHIDIUM

ANONYME — *May and Baker Rev.*, novembre, p. 6, 1955.
BURDIN, M. L. — *Vet. Rec.*, **65**: 241, 1953.
FORD, E. J. H., WILMHURST, E. C., et KARIB, A. A. — *Vet. Rec.*, **65**: 589, 1953*a*.
FORD, E. J. H., WILMHURST, E. C., et KARIB, A. A. — *Vet. Rec.*, **65**: 130, 1935*b*.
LEACH, T. M., KARIB, A. A., FORD, E. J. H., et WILMHURST, E. C. — *J. Comp. Path. Therap.*, **65**: 130, 1955.
MILNE, A. H., et ROBSON, J. — *Vet. Rec.*, **67**: 452, 1955.
ROBSON, J., et MILNE, A. H. — *Vet. Rec.*, **66**: 415, 1954.
UNSWORTH, K. — *Ann. Trop. Med. Parasito.*, **48**: 229, 1954*a*.
UNSWORTH, K. — *Ann. Trop. Med. Parasito.*, **48**: 237, 1954*b*.
THIENPONT, D., et HERIN, V. — *Ann. Soc. Belge Med. Trop.*, **53**: 439, 1955.
WATKINS, T. L., et WOOLFE, G. — *Nature*, **169**: 506, 1952.
WILDE, J. K. H., et ROBSON, J. — *Vet. Rec.*, **65**: 49, 1953.
WILSON, S. G., et FAIRCLOUGH, R. — *Vet. Rec.*, **65**: 201, 1953.
WOOLFE, G. — *Ann. Trop. Med. Parasito.*, **16**: 285, 1952.

C — PROTHIDIUM

BROWNIE, W. M., WATKINS, T. I., et WOOLFE, G. — Notice de Boots Pure Drug Co. Ltd., 1956.
MAGIMEL, J. — Communication personnelle, 1958.
*Rapport Annuel de la Section de Zoologie et de la Tsé-tsé* — Service Vétérinaire du Kenya, 1956.
ROBSON, J., et MILNE, A. H. — *Vet. Rec.*, **69**: 564, 1957.
WATKINS, T. I., et WOOLFE, C. — *Nature*, **178**: 368, 1956*a*.
WATKINS, T. I., et WOOLFE, C. — *Nature*, **178**: 717, 1956*b*.

5° DÉRIVÉ DE LA QUINALDINE: ANTRYCIDE

CHARY, R., et BOCQUET, P. — *Rev. Corps Vet. Armée*, **2**: 13, 1955.
CRUZ FERREIRA, F., et TENDEIRO, J. — Bur. Perm. Interafr. Tsé-tsé Tryp., 115 T, 1950.
CURD, F. H. S., et DAVEY, D. G. — *Nature*, **163**: 89, 1949.
DAVEY, D. G. — *Bull. Off. Int. Epiz.*, **34**: 180, 1950*a*.
DAVEY, D. G. — *Trans. Roy. Soc. Trop. Med. Hyg.*, **43**: 6, 1950*b*.
DAVEY, D. G. — *Vet. Rev. Annot.*, **3**: 24, 1957.
FIENNES, R. N. T. W. — *Brit. vet. J.*, **109**: 280, 1953*a*.
FIENNES, R. N. T. W. — *Brit. vet. J.*, **109**: 330, 1953*b*.
HOARE, C. A. — *Vet. Rev. Annot.*, **3**: 1, 1957.
MORNET, P., LALANNE, A., et CISOOKO, M. — *Bull. Serv. Elev. A.O.F.*, **4**: 7, 1951.
MORNET, P., LALANNE, A., CISOOKO, M., et SIMPSON, S. — *Bull. Serv. Elev. A.O.F.*, **5**: 7, 1952.
NERY GUIMARÃES et PELLIGRINI — *O Hospital*, p. 127, 1953.

PELLIGRINI, D., et BONELLI, G. — *Profilassi*, **24**: 78, 1951.
RAFYI, A., et MAGHAMI, G. — *Bull. Soc. Path. exo.*, **46**: 676, 1953.
*Rapport des Services Vétérinaires de l'Ouganda. 1950 à 1955.*
*Rapport Annuel de la Section de Zoologie e de la Tsé-tsé* — Service Vétérinaire du Kenya, 1955.
*Rapport de Service de l'Élevage du Soudan (A.O.F.), 1955-1956.*
RAY, H. N., SHORT, G. M., SHIVNANI, G. A., et HAWKINS, P. A. — *Ind. Vet. J.*, **29**: 469, 1953.
ROUBAUD, E. — *Bull. Soc. Path. exo.*, **45**: 451, 1952.
SCHOENAERS, F. — *Ann. Soc. Belge Med. Trop.*, **30**: 65, 1950.
SOLTYS, M. A. — *Ann. Trop. Med. Parasito.*, **49**: 1, 1955.
THIENPONT, D. — Bur. Perm. Interafr. Tsé-tsé Tryp., 145, 1950.
TOBBACK, L. — *Bull. Off. Int. Epiz.*, **34**: 157, 1950.
UNSWORTH, K. — *Vet. Rec.*, **64**: 353, 1952.
UNSWORTH, K., et CHANDLER, R. L. — *Ann. Trop. Med. Parasito.*, **46**: 240, 1952.
UNSWORTH, K., et BIRKETT, J. D. — *Vet. Rec.*, **64**: 351, 1952.
VAYSSE, J., et ZOTTNER, G. — *Bull. Off. Int. Epiz.*, **34**: 172, 1950.
WILSON, S. G. — *Vet. Rec.*, **61**: 397, 1949.

### 6° ASSOCIATIONS DE TRYPANOCIDES

CHAUMARD, R. — *Rev. Corps. Vet. Armée*, **1**: 10, 1955.
FINELLE, P. — Thèse Doctorat Vétérinaire, Alfort, 1952.
LAUNOY, L. — *C. R. Soc. Biol.*, **107**: 1498, 1931.

### 7° COMPLEXES DE SURAMINE

DESOWITZ, R. S. — *Ann. Trop. Med. Parasito.*, **51**: 457, 1957.
MAGIMEL, U. — Communication personnelle, 1958.
WILLIAMSON, J., et DESOWITZ, R. S. — *Nature*, **177**: 1074, 1956.
WILLIAMSON, J. — *Ann. Trop. Med. Parasito.*, **51**: 440, 1957.

### 8° CHIMIORÉSISTANCE

DAVEY, D. G. — *Bull. Off. Int. Epiz.*, **34**: 180, 1950.
DAVEY, D. G. — *Vet. Rev. Annot.*, **3**: 15, 1957.
FIENNES, R. N. T. W. — *Vet. Rec.*, **64**: 423, 1952.
FIENNES, R. N. T. W. — *Ann. Trop. Med. Parasito.*, **44**: 222, 1953.
RICHET, P. — *Bur. Perm. Interafr. Tsé-tsé Tryp.*, p. 72, 1954.
SCHNITZER, R. J., et GRUNBERG, E. — *Drug resistance to microorganisms.* Acad. Press. Edit. New-York, 1957.
UNSWORTH, K. — *Ann. Trop. Med. Parasito.*, **48**: 178, 1954.
WILSON, S. G. — *Congrès Int. Med. Vet. Stockholm*, **1**: 12, 1953.

# TSETSE FLY RECLAMATION AND DEVELOPMENT IN EASTERN AFRICA

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In the early years of the exploration of tropical Africa, the consequences of exposing domestic animals to attack by tsetse fly were soon realised, although it was thought that mortality was caused by poisons injected by the tsetse fly when feeding. It is worth noting that Livingstone (1), about to explore the Zambezi River in its course through Mozambique, was instructed, in 1858, by the great anatomist Richard Owen to determine «with the utmost possible precision [...] the number of bites or punctures occasioning death». This attempt to initiate a study of the epidemiology of nagana bore no fruit and almost the full century had to pass before the peculiarities of antrycide as a prophylactic demanded estimation of the «trypanosome challenge» as a preliminary to field use; a problem, in one of its aspects, of the numbers of tsetse fly bites the host animal receives.

Two great biological catastrophes focussed attention upon the tsetse fly and the need for its control. The common coincidence of the larger fauna and the tsetse fly was apparent to many of the early pioneers in Southern Africa and when, in 1896-97, the mortality in both wild fauna and domestic cattle, caused by the Great Rinderpest, was followed by the widespread disappearance of tsetse fly in the Transvaal and Rhodesia, it became clear that destruction of the wild game offered a means of eradicating the insect.

The second catastrophe was the great epidemic of Gambian Sleeping Sickness, five years later, on the shores of Lake Victoria.

This event provided the first major stimulus to research both on the trypanosomes and their vectors. Control of the Uganda epidemic was effected by removal of the survivors from contact with the tsetse and, in places, by destruction of the lake and river-side forests and thickets which formed their habitat. Both processes, no doubt, were assisted and made more obvious by the close adherence of *Glossina palpalis* to the water's edge.

The third basic method of eliminating *Glossina*, by direct attack on the insect itself, first appears in the invention in 1906 of Sr. Maldonado, a farm manager on the Island of Príncipe, where, between 1911 and 1914, some half-million *Glossina palpalis* were trapped on adhesive cloths worn on the backs of African labourers. It was, however, concluded by da Costa that the method could never alone exterminate a tsetse population and its chief use was to provide an index of the effectiveness of other measures, a conclusion which may now be regarded as valid for trapping methods of all kinds. It was not until the post-war manufacture of various insecticides that direct attack on the individual tsetse flies, by any means, could be contemplated on a large scale (2).

Although the Island of Príncipe lies, geographically, outside the scope of this paper, I may be forgiven, especially in Lisbon, for considering the work of da Costa and his colleagues in exterminating its population of *Glossina palpalis* and eradicating sleeping sickness. They had peculiar advantages, it is true. The area of the island was 126 $km^2$ (49 sq. miles) and it was separated from the nearest source of reinfestation by *Glossina* by 200 km (125 miles) of open sea. They were also supported by extremely comprehensive legislation. The feature of the operations which we ought to notice here was their very wide scope, embracing, as they did, almost every method of attack known to-day, both on the trypanosome and on the vector. The latter was attacked by destroying the wild hosts by hunting; by destruction of the habitat by tree and thicket felling; and, as noted above, by direct extermination of the individual insects by the Maldonado method, which provided one of the earliest examples of the use of «fly-boys» as a means of measuring changes in the tsetse population density. Further, in order to facilitate control measures, extensive drainage works were undertaken. The principle of separating the vector from its potential hosts was also invoked in order to

prevent the spread of trypanosomes. Houses were gauzed; dogs, recognised both as carriers of the trypanosomes and as a food supply for *Glossina* were supposed to be confined to their owners' houses during the hours of daylight: native labour was provided with protective clothing, long-sleeved and long-trousered. Finally, extensive examinations by thick blood films were periodically undertaken of the whole population at risk, sufferers from sleeping sickness were isolated and treatment, both therapeutic and prophylactic, with atoxyl, was administered. In three and a half years *Glossina palpalis* was exterminated and the disease virtually extinguished.

I have dealt with the campaign in Príncipe at some length becaute it illustrates a very sound principle, namely that of attacking both the disease and its vectors at every possible point: a principle which, in British colonial territories, tended to be overlooked, especially where veterinary trypanosomiasis was concerned and where reliance was placed almost entirely upon extermination of the vector, often by one method to the exclusion of all others.

It was only ten years ago that the appearance of antrycide, a drug which had prophylactic properties especially against *Trypanosoma congolense* infections, radically changed the whole outlook on the problem, altering it at once from a basically entomological problem to one fundamentally epidemiological. The effectiveness of antrycide depends upon the «trypanosome challenge», now conventionally measured as the product of the infection rate of trypanosomes in the tsetse population and the latter's apparent density. Antrycide fast strains of trypanosomes are liable to appear under faulty dosage regimes and it is therefore necessary to have in reserve one or more curative «stopper» drugs to which antrycide fast trypanosomes are not tolerant. Prophylactic drugs have two main uses. They allow cattle to be grazed in fly belts where the challenge is either naturally low or artificially reduced by attack on the tsetse fly. They also offer protection to cattle populations confronted by spread of tsetse fly so that there is no need to evacuate pastures and time is gained for effective control measures to be introduced. The need for antrycide to be administered every two months may, in remote areas, place a great strain upon the administrative resources of veterinary departments, but there is every probability that drugs giving longer periods of protection will soon be in wider use.

We ought also to note another benefit derived from the use of drugs. In the Sudan and also in Uganda, antrycide, as a curative drug, has been used with marked effect to clean up widespread enzootic trypanosomiasis hitherto kept at a high level of incidence by mechanical transmission.

We may now discuss certain changes in ideas about the tsetse problem which have occurred as new techniques and wider knowledge of African ecology became available.

The first big attempt to eradicate tsetse fly was made in Southern Rhodesia by Jack and Chorley, whose work is still unsurpassed as the largest achievement in tsetse reclamation, since, by 1950, some 10 000 square miles had been reclaimed from *Glossina morsitans* at an average cost of about sixpence per acre. (At present day rates this is nearer to Shs. 1/6, but is still considerably cheaper than most other methods in common use.) In Uganda, during the last ten years, the same method has also produced eradication, both of *Glossina morsitans* and *G. pallidipes,* on a very large scale and at a low cost compared with other methods.

It was the desire to preserve the wild fauna of Africa that inspired Swynnerton, a farmer in Rhodesia, to attempt to develop methods of modifying the natural vegetation as alternatives to the slaughter of game animals then beginning in that country. We need not follow the arguments for one method against the other, except to note, in favour of game destruction, that it not only leads to the elimination of *Glossina,* but also to the elimination of the natural reservoir of the pathogenic trypanosomes.

Another controversy arose, in British colonial spheres, when Swynnerton began to develop his Department of Tsetse Research in Tanganyika. Insufficient funds being available for research to be adequately promoted upon all aspects of the trypanosomiasis problem, a dispute began as to whether it was wise to assume that its solution was to be sought only in eradicating the vector, since research on the trypanosomes and the pathology of trypanosomiasis might provide other answers to the problem. Out of this dispute arose another. The view began to be advanced that *Glossina* had been, for thousands of years, the guardian of the African soil against destruction by primitive forms of agriculture and animal husbandry. Was it wise to remove this powerful agent of biological control, to allow the native

cattle of Africa to multiply unchecked and so to hasten the day when the sands of the Sahara and of the Kalahari should meet? Perhaps the most valuable feature of this dispute was the gradual realisation that the presence of the tsetse fly was not the only barrier to occupation of the many thousands of square miles of empty wooded grasslands and thorn savannah. Much of the tsetse infested area was waterless for a large part of the year; its cattle population, in the event of reclamation and even if water could be provided, must be very carefully controlled if rapid over-stocking were not to develop, while agriculturally no alternative to shifting cultivation by small communities suggested itself. In short, the tsetse problem was only one feature of the vastly more complex problem of how to develop the empty lands in such a way that a profitable and, above all, a stable rural industry could occupy them. In the event, the protagonists of the view that extermination of all tsetse flies as rapidly as possible should be the main objective of all glossinologists, while others might look after the preservation and development of the African soil, if they wished, were defeated. Since the British African Conference on Land Utilisation in 1949 (3) the view has prevailed in British colonial territories that tsetse ought only to be eradicated from areas scheduled for early occupation and development. It should be noted here that this policy had been formulated in Portuguese territories some years earlier (4). One may also observe that the victory of those who believed that tsetse reclamation should go hand in hand with rural development was achieved not by advancing the plea for *Glossina* as the guardian against soil erosion, but by the demonstration that, unless the land reclaimed was occupied in such a way that the natural vegetation was suppressed and the wild fauna excluded, the tsetse fly would return and money spent would be wasted.

The other dispute, as to whether work on the vector should be promoted to the exclusion of research on the trypanosomes, had already been settled by 1947 when the fact finding surveys of Profs. P. A. Buxton and T. H. Davey led to the establishment in East and West Africa of institutes in which research on trypanosomes and trypanosomiasis would be expanded until it equalled, if it did not surpass, in volume, research on the vector. But undoubtedly, the most powerful stimulus to research, especially on the cattle trypanosomes, was provided by the discovery of antrycide, the prophy-

lactic properties of which offered the possibility of controlling nagana without the need to eradicate the vector.

The relationship between the problems of overstocking and of tsetse infestation has been much clarified since the Land Utilization Conference resolutions were passed in 1949. It was then recommended that «reclamation of large tsetse infested areas for African owned herds maintained on a primitive pastoral system of management would be uneconomic». But it was already clear to some that this did not provide a basis for a workable policy. Thus, Hornby, at the Goma Soils Conference in 1948 (5), recalling that he had often said he would only remove tsetse where he was convinced that stock density on the reclaimed land would be controlled, now concluded that tsetse «have contributed to the conservation of the soil of large areas at the cost of more intense erosion of other large areas and their implement of conservation has been death of men and animals». He pointed out that the first stage of soil rehabilitation must be a wider distribution of populations and. to achieve this, large scale reclamation was necessary and desirable.

The magnitude of the problem is apparent from a number of surveys of natural resources potential which have appeared in British territories (and, no doubt, in others) during the last five years. The East Africa Royal Comission (6) used as a simple guide to rural economic prospects the expectation probabilities of rainfall, but pointed out that this was not the sole agent restricting agricultural expansion. Other factors listed included, first, tsetse infestation, then shortage of water supplies, inadequate communications, lack of man power, condition of the soil and disease. From the Royal Commission's maps one percives that only on 17 percent of the land surface of East Africa is arable farming likely to provide a sound basis for rural industry. The rainfall on 48 percent of the three territories will not normally permit of arable farming, while of the marginal 35 percent remaining, two-thirds have only a poor prospect of success in this direction.

We may now examine these land categories in relation to the tsetse belts with which they only in part coincide. Of the total land surfaces of East Africa (640.080 square miles) some 280,000, or 43 percent, are infested by *Glossina*. We find that the categories of land as defined by rainfall potential within the tsetse belts are some-

what different in their proportions from those obtaining in the East African territories as a whole. Land with a good prospect of obtaining 30 inches of rain, and, therefore, suited to arable farming, forms only 10 percent of the tsetse infested areas; land with a bad prospect of receiving 30 inches, which will not permit of arable farming, forms 34 percent of the tsetse belts. We may note in passing that in East Africa as a whole it would appear that some two-thirds of the land which is too poor for arable farming is also too poor to support *Glossina* and, indeed, the larger part of it falls within the desert or semidesert of the northern and eastern portions of Kenya. We may also note that of the favourable land still tsetse infested, the largest portion lies in the Kilombero valley of southern Tanganyika and we may suppose that the main obstacles to its utilization hitherto have been the failure of the indigenous inhabitants to recover from the depopulation of fifty years ago and to lack of communications. We may also suppose that when these deficiencies are removed (by provision of adequate development capital), the tsetse problem in this valley will prove of trivial importance. It is, of course, with land that is either marginal or poor or bad that the glossinologist must usually concern himself. Moreover, this trend will become more pronounced and he will, of necessity, have to work in increasingly poorer and poorer lands capable only of supporting rural industries (usually pastoral) which are useless from the point of view of consolidating the achievements of tsetse reclamation. We must note the extreme consequence of this situation. The recent report on Agricultural Policy in the Federation of Rhodesia and Nyasaland (7) notes that «there are cases where the soundest use of land is to do nothing with it». And it is evident from the most cursory examination of much of the Zambezi Valley tsetse belt in Southern Rhodesia that land on which it would be possible to settle people with any prospect of their being able to subsist unaided in contact with peripheral tsetse is extremely limited. One must either become reconciled to the persistence of *Glossina* into an indefinite future or it must be eradicated regardless of whether the reclaimed lands have any potential for use. Here we should add that, provided it can be enforced, legislation may supply an alternative to the tsetse as an agent of soil conservation.

The point made above, that as he progresses and achieves success the anti-tsetse worker must necessarily operate in poorer and poorer

country is of some importance. This is especially so where we are dealing with the problem of over stocking where it exists on a large scale. In 1913 the cattle population of Tanganyika was given as 1,700,000 head (8). How reliable this figure is one can only guess, but it is certain that at that time the cattle population was still recovering from the Great Rinderpest. By 1925 a census showed nearly 4 ½ million, which increased to over 5 million by 1938 and this, in turn, in the next ten years, to nearly 6 ½ million head. In 1949 well over half a million head died of thirst and starvation, but by 1952 the population had recovered and has continued to increase, reaching 6 ½ million in 1955. (We need not complicate the picture here by considering about an equal number of sheep and goats.) During the period 1938 to 1955 the total increase in cattle population was well over 1 ½ million or a rate of nearly 100,000 head per annum, in spite of increasing marketing of stock, which had reached 200,000 head in 1948 and, by 1955, over a quarter of a million.

We have now reached a point at which we can attempt to summarise the main issues in number of separate tsetse problems. We have seen that the situation in Southern Rhodesia is, to some extent, controlled by the fact that a part of the tsetse infested land is best dealt with by leaving it alone. (Leaving it alone does not, of course, exclude turning such areas into game parks or forest reserves.) The decision therefore has to be made as to whether one should attempt to eliminate the whole fly belt (which is of a size susceptible to such an attempt) or whether one should look forward to an indefinite period of heavy recurrent expenditure on tsetse defence measures and the widespread use of prophylactic and curative drugs. In fact the decision has for long been made in favour of a policy directed towards the total elimination of *Glossina*.

In the Tanganyika situation, however, where the fly belts are so large that total eradication in the foreseeable future is out of the question, the problem is very much more complex. It would seem, from the figures quoted above, that, in its most elementary form, it resolves itself into a demand for pasture for an additional 100,000 head of cattle annually. If, as it appears, this must be sought in somewhat poorer land than that in which the parent stock are pastured, we cannot expect to improve, at present, upon a beast to 10 acres and the demand must be for about 1,500 additional square miles per

annum, or at least five times as much as is currently being reclaimed in that territory.

In Kenya (9) the problem has been defined, at least in some areas, and is being tackled on a basis of the present pasture deficiencies. This has been possible for four reasons. One, that control of cattle trypanosomiasis (including vector control) is in the hands of the Veterinary Department, which is also concerned with pasture management. The second is that the fly belts are either infested with *G. pallidipes* or *G. austeni* and can be tackled on a smaller scale than can be belts of *G. morsitans* in Tanganyika. The third reason is that extensive use, based on a well organised epidemiological service, is made of prophylactic and curative drugs. The fourth reason is that geographically it has been possible to divide up the problem as a whole into a relatively large number of administratively separate district development schemes.

The Tanganyika problem is, in fact, the very largely administrative and logistic one of applying to the *morsitans-swynnertoni* country, on the one hand, and, on the other, to the vast single cattle populations of Usukuma, Ugogo and Masai, the approach apparently so successfully being developed in the *pallidipes* and *austeni* belts of Kenya.

In order to complete the East African picture, Uganda may be mentioned. Here is yet another situation not unlike that of Southern Rhodesia. The similarity is due, in some respects, to both countries having applied, with success, the technique of tsetse eradication by game destruction, with a result that both have extensive areas of artificially fly-free country which must be protected from reinvasion. In contrast to Tanganyika, Uganda has every reason, at present, to encourage the growth, as well as the improvement, of its stock population (as has Rhodesia) and, again, because of the peculiarities of its local trypanosomiasis problem, modern techniques are making this possible. In the somewhat humid climate of central Uganda, haematophagous insects other than tsetse are abundant (particularly *Stomoxys*) and mechanical transmission of trypanosomes is frequent. Thus, in large areas free of *Glossina* there was abundant enzootic trypanosomiasis. The use of block treatment, mostly with antrycide methyl sulphate, from 1953 onwards, has done much to eliminate

this form of the disease with consequent marked increases in local cattle populations (10).

Another similarity between Uganda and Southern Rhodesia lies in their geographical situation *vis-à-vis* the major tsetse belts; Rhodesia lying near the southern extremity of the great *morsitans* belt of eastern Africa, Uganda lying between the northern limits of this belt and the eastern limit of the *submorsitans* belt across the southern boundaries of the Sahara. From their geographical and ecological positions it is not impossible that both territories may, within a not too far distant future, reduce their trypanosomiasis problems to the task of defending their frontiers from tsetse invasion from their more heavily infested, less fortunate, neighbours.

We may sum up by noting that there now exist a number of methods by which *Glossina* may be controlled or eradicated. The trypanosome diseases, human and animal, are curable and also, to an increasing extent, susceptible to treatment by prophylactic drugs. The main problem of the practical worker in the field still remains to understand the status of the diseases, both sleeping sickness and nagana, in African ecology and economics and to select and apply the treatment appropriate to a wide variety of local situations.

## BIBLIOGRAPHY

1. Wallis, J. P. R. (Editor.) — *The Zambezi Expedition of David Livingstone, 1858-1863,* Appendix N° 2, London, 1956.
2. Bruto da Costa, B. F., Sant'Anna, J. F., Santos, A. C. dos, and Araújo Álvares, M. G. — *Sleeping Sickness. A record of Four Years War against it in the Island of Principe.* Centro Colonial, Lisbon, 1916
3. *Final Report. Bristish African Land Utilization Conference, Jos, Nigeria, November 7th-15th, 1949,* n. d., Zaria, Nigeria.
4. Sousa, J. de — Conferência Intercolonial sobre Tripanossomíasis, Lourenço Marques, 26 a 31 de Agosto de 1946. *Address on distribution of tsetse fly in Mozambique,* 1947.
5. Hornby, H. E. — «Tsetse Problems in Relation to those of Soil Conservation.» Conference Africaine des Sols, Goma, Congo Belge. *Bull. agric. du Congo Belge,* xl, 1948.
6. *East Africa Royal Commission, 1953-1955 Report.* Cmd 9475, H.M.S.O., London. 1955.
7. *Agricultural Policy in the Federation of Rhodesia and Nyasaland. Report to the Federal Minister of Agriculture by the Federal Standing Committee on*

*Agriculture Production in collaboration with Professor Sir Frank Engledow, C.M.G., F.R.S.* C. Fed. 77, Salisbury, Southern Rhodesia, 1958.

8. HILL, J. F. R., and MOFFETT, J. P. (Editors) — *Tanganyika. A review of its resources and their development.* Goverment of Tanganyika Territory, London, 1955.
9. *Annual Report for 1956 of the Department of Veterinary Services, Kenya, Part V, Zoological Services.* Nairobi, 1957.
10. *Annual Report of the Department of Veterinary Services and Animal Industry for the year ended 31st December, 1954.* Entebbe, 1955.

# THE CULTIVATION *IN VITRO* OF *TRYPANOSOMA BRUCEI, TR. CONGOLENSE, TR. CRUZI, TR. GAMBIENSE* AND *TR. RHODESIENSE* AND THE BIOLOGIC AND DIAGNOSTIC SIGNIFICANCE OF THE CULTURES

ARDZROONY PACKCHANIAN

Five species of pathogenic trypanosomes were successfully grown *in vitro* on slightly modified Novy and MacNeal's (NN) medium, containing 50-75 % defibrinated rabbit blood. The chief difference between this «modified medium» as compared with the original NN medium consisted of the use of double infusion beef meat (cold and hot extraction), NaOH instead of $Na_2CO_3$ for adjusting pH, and Difco peptone instead of Witte's peptone. For convenience, this so-called modified medium is referred to as NNP (Novy, MacNeal and Packchanian) medium.

Three Uganda strains of *Tr. brucei* were grown on NN and NNP mediums from 70 experimentally infected animals (3 rabbits, 4 guinea pigs, 26 rats, 35 *Mus musculus* and 2 *Peromyscus maniculatus gambeli*) during 1954 to 1958 without a single failure. Two strains of *Tr. congolense*, one from the Wellcome Laboratories of England and the other from the Belgian Congo, were grown on NNP medium successfully from 10 infected animals, 1 guinea pig and 9 mice. Four strains of *Tr. gambiense* from the Belgian Congo, one of which was arsenic-resistant, and spinal fluid from one patient with sleeping sickness all yielded positive cultures of trypanosomes on NNP medium. Two strains of *Tr. rhodesiense* from the Belgian Congo invariably gave positive culture of flagellates on NNP medium in 10 experiments.

*Tr. cruzi* was grown on NNP and NNP-2 (1.3 % beef infusion agar containing 20-40 % defibrinated rabbit blood) 52 times from human cases of Chagas' disease, and over 400 times from naturally and experimentally infected animals. Subcultures were made monthly on NNP or NNP-2 media from each species of trypanosome from 6 months to as long as 30 years. After several subcultures the flagellates grew not only in the water of condensation, but also colonized on the slant portion of the medium.

Present and past studies indicate that whenever a given species of trypanosome is transmittable to either man or animals by insect vectors, they can readily be grown *in vitro* on NN or NNP or similar media. Thus the cultural test *in vitro* from man or animal is highly significant and diagnostic for trypanosomiasis. Often positive cultures were obtained from man and animals when direct microscopic examinations of their blood were negative for trypanosomes.

The morphology of flagellates *in vitro* of each species of trypanosome possessed certain characteristics which facilitated their identification. Biologic, immunologic and immunochemical studies also have revealed several characteristics distinctive to each species.

# THE MEASUREMENT OF ANTIBODY OCCURRING IN HUMAN AND BOVINE TRYPANOSOMIASIS BY A RESPIROMETRIC METHOD

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A research program at the West African Institute for Trypanosomiasis Research has been directed toward elucidating the nature of the resistance to trypanosomiasis possessed by some breeds of cattle, such as the N'dama and Muturu. In conducting this study it was found that existing techniques employed to measure the immune reaction in trypanosomiasis were not sufficiently sensitive or reliable. The technique finally used, and described here, is based upon the observation that antibody reduces the respiratory rate of trypanosomes. Furthermore, the degree of reduction is proportional to the antibody titre; thus a quantitative estimate of titre can be obtained.

The procedure for this «respiratory test» is as follows: The test is carried out in a conventional Warburg apparatus. The temperature is set at 36 5°C. Trypanosomes are obtained from the heart blood of heavily infected rats. Sodium citrate is used to prevent coagulation. Glucose is added to raise the blood sugar level to approximately 350 mg %. This is done to exclude an extrinsic decrease in oxygen consumption due to glucose deprivation. To two 20 ml volume Warburg flasks, 2 ml of serum to be tested for antibody is added. Also added into two flasks is 2 ml of control serum from an uninfected animal. The center wells are filled with 20 % NaOH solution. Into each flask an identical amount of infected blood is pippetted.

This amount varies from 0.1 ml to 0.25 ml, depending upon the parasite density. The flasks are placed on the manometers, immersed in the bath, and after 10 minutes equilibration the system is closed. Readings are taken every 10 minutes for one hour. The oxygen consumption of the trypanosomes suspended in the control serum is compared with that of those in the sera being tested for antibody. From this comparison the antibody titre, expressed as a numerical index, is obtainable. If no antibody is present, the trypanosomes suspended in the test serum will show the same oxigen consumption as those in the control serum. The index in this case is, therefore, 0. In the other extreme instance where a large amount of antibody is present no oxygen is consumed and the index is 100. The index can be calculated by the formula:

$$\text{Antibody index} = \frac{\text{mm/302 hr/trypanosomes in test serum}}{\text{mm/302/hr/trypanosomes in control serum}} - 100\,\%$$

The antibody appears to be species specific, but not strain specific. In the case of *T. vivax* at least, the strain adapted to the rat host is considerably more sensitive to antibody produced by bovines than the «normal» strains maintained in sheep or cattle by cyclical passage through tsetse. A serum sample obtained from a highly resistant N'dama gave an antibody index of 100 when tested against the rat adapted strain, but an index of only 60 when tested against the strain maintained in sheep.

The two following examples exhibit the different changes in antibody titre following challenge with the same tsetse transmitted strain of *T. vivax*. Fig. 1 depicts these progressive changes in a Zebu cow, a breed normally susceptible to infection. Shown graphically in this figure are the course of parasitaemia, changes of antibody index and changes of serum gamma-globulin content as determined by Antweiler micro-electrophoresis. Trypanosomes were first seen in the blood 7 days after challenge; a series of intense parasitic attacks ensued. There appears to be a definite relationship between the course of infection and the fluctuations in antibody index. The increasing parasitaemia is accompanied by a coincident rise in antibody index. This is followed by crisis with a rapid decrease, or apparently complete disappearance of trypanosomes from the blood. The high anti-

body level is not sustained and, with the fall of parasite numbers, it rapidly diminishes. It is interesting to note that the index variation is roughly proportional to the intensity of the parasitaemia. During the first 19 days of infection, when the parasitic attacks were se-

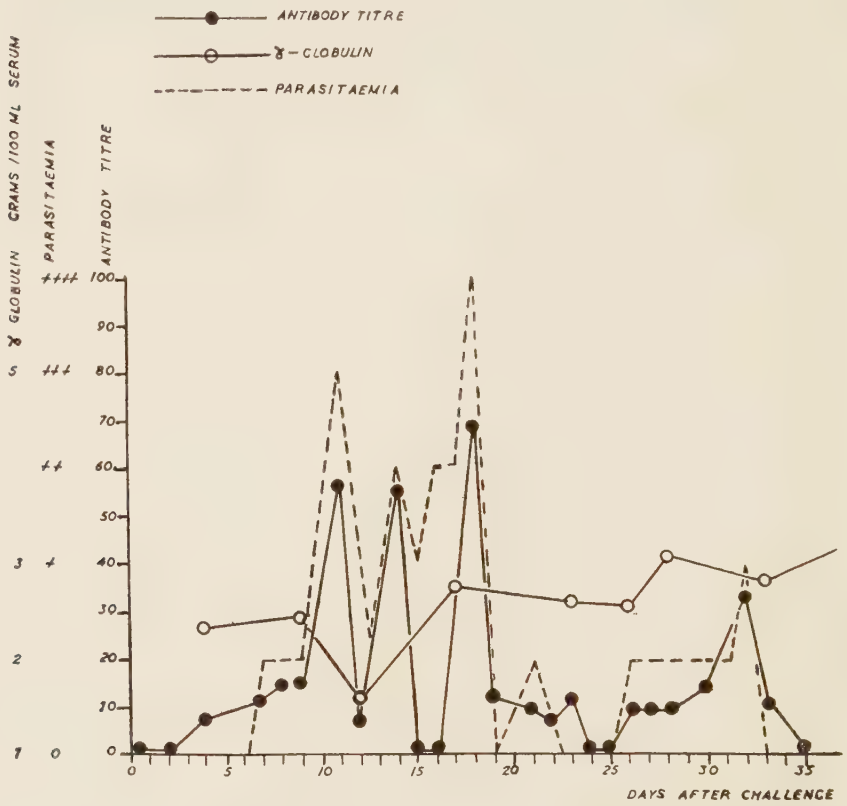


Fig. 1 — The immunological response of a Zebu cow following challenge with *T. vivax*

verest, antibody index rose to its highest value of 69 on the 18th day. Thereafter the lower parasitaemias were accompanied by lower titres. A variation in gamma-globulin level was observed during the course of infection. The peak, 3.10 g/100 ml serum, occurred on the 28th day.

The other example, shown in Fig. 2, depicts the distinctly different immunological response of the N'dama breed. Parasites appeared,

in very scanty numbers, only on the 9th day following challenge. After this time no parasites were again seen during the 7 months that daily blood films were examined. Antibody was not detectable previous to challenge, but an index of 8 was noted by the 2nd day

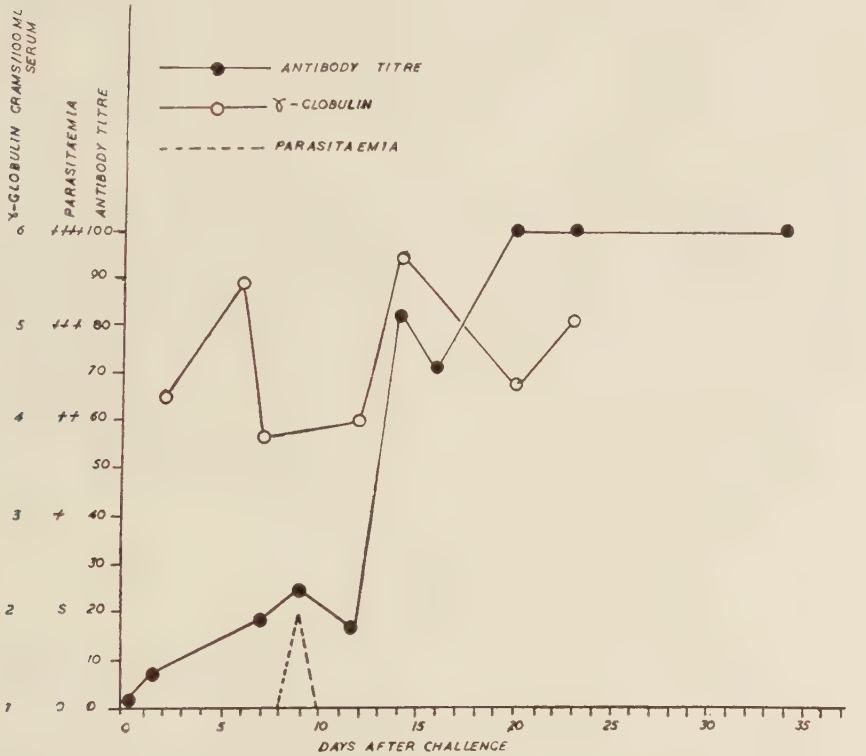


Fig. 2 — The immunological response of a N'Dama cow following challenge with *T. vivax*

and one of 25 by the 9th day. Crisis occurred and antibody index fell to 16 on the 12th day. Thereafter, titre rose sharply, to 83 by the 14th day and 100 by the 20th day. Trypanolytic activity is truly remarkable at the highest titre; there is no respiratory activity, the trypanosomes being lysed almost immediately upon being mixed with the serum. This very high titre is sustained for a considerable period. An index of 100 was still noted on the 86th day after chal-

lenge. By the 100th day titre had fallen slightly to 96.3, and to 61.8 by the 131st day.

The picture presented by the serum gamma-globulin level of the N'dama is also unique when compared to that of the Zebu. The initial level was much higher than that found in the Zebu, 4.2 g/100 ml, as compared to 2.38 g. This initially high level fell slightly between the 7th and 12th days, i.e., during the brief patent period, but rose again to its previously high level after this time.

Studies on the immune reaction occurring in human trypanosomiasis have only recently begun. However, there is already promise that the respirometric method of measuring antibody will prove to be a useful research tool in this area. In the first series of 18 patients known to be infected with *T. gambiense*, 17 exhibited antibody indices ranging from 3.5 to 84.2 when their sera were tested by this technique. The test organism employed in this experiment was a strain of *T. gambiense* adapted to white rats.

# THE CONTROL OF TSETSE FLIES WITH INSECTICIDES

K. S. HOCKING

Tsetse flies, *Glossina* spp., are widespread in tropical Africa, where many species are important vectors of trypanosomiasis of man and of animals. The usual methods of control are based on partial or total clearance of the bush habitat of the fly and suggestions for control by chemical means must show some advantage, such as in cost or in speed of action, over these usual methods. In all methods of control by insecticides, isolation of the tsetse habitat is essential for a permanent result, but not, of course, necessary if only a short term reduction in numbers is required.

## APPLICATION BY AIRCRAFT

Some species of tsetse flies, such as *G. morsitans, G. swynnertoni* and *G. pallidipes* in East Africa, occur over wide areas of country, often many hundreds of square miles in extent, and, for the control of such species, the use of aircraft for the application of insecticide has obvious advantages. Against the more confined riverine species such as some *G. palpalis,* ground methods of application may be adequate and cheaper. Application from the air has been found to be uneconomic, even if possible, against species living in high forest, such as some *G. palpalis* and *G. brevipalpis* (1).

Savanna woodland, even if containing evergreen thickets, can be successfully treated; though it is preferable that the leafless period of the year is chosen. The only effective applications have employed the insecticide in the form of a coarse aerosol containing as much as possible of its volume in droplets between 5 $\mu$ and 50 $\mu$ in diam-

eter. Larger droplets are unable to by-pass obstacles such as leaves and branches and penetrate to the resting sites of the tsetse flies and smaller droplets do not deposit on the insects.

Under tropical conditions, applications of coarse aerosols are effective only for a limited period just after dawn and unreliably for an even shorter period before dusk. At other times meteorological conditions are so unstable that rapid diffusion of the aerosol occurs (2).

Aerosols have been produced from aircraft emitting an oil solution of insecticide under high pressure through fine nozzles on an extended boom, by injection of the insecticide solution into the exhaust of the aircraft, or by various types of rotary atomisers. Injection into the exhaust system involves some decomposition of the insecticide by heat; fine enough droplets can be produced by the first method only if used on a reasonably fast aircraft, and the most promising equipment for aerosol production from light aircraft would seem to be the Micronair spinning cage produced by Messrs. Britten-Norman Ltd.

Using larger aircraft (the Avro Anson) a suitable aerosol was produced from an oil solution of insecticide emitted under a pressure of 150 lbs per square inch, through Bray nozzles pointing forwards, with an air speed of approximately 120 m.p.h. An effective procedure has been to use a 10 % solution of DDT in oil at one quart per acre (1 l per 0.4 ha) with a swathe width of 50 m. Eight such applications at fortnightly intervals have eradicated *G. morsitans* and *G. pallidipes* from a 16 square mile block at a cost of about £ 1,000 per square mile (3) and radically reduced the tsetse population in other cases (4). Experimentally, a 5 % solution of BHC in oil is now being applied at a dosage of 1/12 gallon per acre using an Auster aircraft, fitted with the Britten-Norman Micronair equipment.

Recent calculations (5) have shown that, with applications giving a 90 % kill of adults, monthly intervals between applications should be as effective as 2 week intervals. This enables a light aircraft to be used for larger areas and an attempt is to be made to eradicate *G. morsitans* and *G. pallidipes* from a 12 square mile block in this way at a greatly reduced cost.

One interesting point arising from our experiments was that, if a tsetse habitat was completely isolated from surrounding fly bush

and the tsetse population in it reduced to a low level, the population did not increase again over a period of several years. Thus, even without complete eradication of the fly, time is allowed for the inevitably slow occupation of the bush by settlers, before the risk of trypanosomiasis in their cattle is sufficient to discourage them,

## GROUND AEROSOL MACHINES

Under this head can be included machines such as the T.I.F.A. and the «Swingfog», which use a blast of hot air to produce aerosols, and canisters, which produce insecticidal smokes by the burning of combustible mixtures containing insecticides.

Experimentally and on a limited scale, smoke canisters have been used with some success by us against *G. palpalis* in Uganda and by E.A.T.R.O. against *G. swynnertoni* in Tanganyika (7). The chief difficulties in their use are connected with getting adequate and uniform distribution of the smoke under varying wind directions and meteorological conditions.

Ground aerosol machines could compete directly with aircraft applications for the destruction of savanna tsetse species, but would need a more elaborate organisation to cover an extensive area. They have so far been used for special purposes only. The «Swingfog» has been tried in Rhodesia to prevent the carriage of tsetse flies by road traffic and in Kenya the T.I.F.A. has been used do disinfest trains in a similar way.

## RESIDUAL TREATMENT OF VEGETATION

*a*) In East Africa the riverine tsetse *G. palpalis* is apparently dependent on the water's edge vegetation for its feeding area. Treatment of this vegetation with an emulsion of a residual insecticide, so that it remains lethal to the fly, on short contact, for more than a pupal period (say for 2 months), is generally sufficient to control this species and possibly to eradicate it. This method was developed by this Unit in 1948 (8) and adopted by Kenya as a control measure from 1951 (9). The earlier control measures of this nature were

carried out with 5 % DDT emulsion applied at about 15 gallons per mile of river, four times at two-weekly intervals, which cost about £ 42 per mile. It was later found that 1.5 % dieldrin emulsion applied twice with a month interval, or 5 % dieldrin emulsion applied once, both at the same rate, were more effective. In Uganda the standard method is now to apply 5 % dieldrin emulsion once only to selected portions of the riverine vegetation at a total cost of £ 20 per mile. These costs compare with an average cost of bush clearing on these rivers of £ 200-300 per mile. In most of this control work the insecticide has been applied with knapsack or compression sprayers by labourers walking down the river bed, but in some larger streams a more effective method has been found to be application by a power sprayer from a punt which is polled down the river.

*b*) A considerable amount is known about the resting places of the savanna species of tsetse fly in East Africa, i.e., *G. morsitans* and *G. swynnertoni*. In a block of 15 square miles of isolated *G. morsitans* habitat in Uganda an experiment is being carried out where dieldrin emulsion is being applied to the lower sides of the branches of *Acacia hebacladoides* trees in fly concentration areas. About 40 gallons of 20 % concentrate is being used per square mile and as a control measure the total cost would probably be about Shs. 6/- per acre. The effectiveness of the method is, however, not yet determined.

In areas with a very severe dry season *G. morsitans* becomes an effectively riverine species, and in the North of the Northern Region of Nigeria, for example, where the relative humidity is down to about 5 % for most of the day during several months of the year and the maximum temperatures are well over 100°F, this fly is confined during the heat of the day to the lowest part of the trunks of the well shaded trees along th river banks. Treatment of these places with residual insecticide, which is a comparatively cheap and rapid procedure, is sufficient therefore to eradicate *G. morsitans*. The Veterinary Department of the Northern Region of Nigeria is treating effectively areas of the order of 100 square miles in a season at a total cost of about Shs. 5/- per acre (10). In places where the dry season is neither so prolonged nor so severe, more extensive treatment is required, but the method is still practicable.

## OTHER METHODS OF CONTROL

Attempts have been made to control tsetse flies by the use of treated traps and treated attractive screens both in East and West Africa. Eradication has not been achieved, and is not likely, by these means, but they may have their use in the reduction of tsetse numbers in such places as river crossings and watering places in sleeping sickness areas. Attemps have been made in Kenya to improve the efficiency of the method by the addition of attractants to the traps. If successful, the development of attractants might also be of value in increasing the contact between the fly and treated stretches of vegetation. Conversely repellents might be of use in enabling cattle to cross narrow fly belts without acquiring trypanosomiasis.

An interesting control method which has been tried experimentally and shown promise is the use of insecticidally treated cattle as lethal bait for tsetse flies (11, 12). The main difficulties in the method are dispersing the cattle in such a way that a high proportion of flies contact them for food rather than the natural game hosts in the area, and also keeping the insecticide deposit of the cattle at a lethal level for a sufficient length of time. Attempts have been made to solve the second difficulty by feeding insecticides to cattle and thus making their blood toxic to blood sucking insects, but this is not yet a practicable proposition.

## BIBLIOGRAPHY

1. Hocking, K. S., and Yeo, D. — *Bull. ent. Res.*, **44**: 589-600, 1953.
2. Yeo, D., and Thompson, B. W. — *Bull. ent. Res.*, **45**: 79-92, 1954.
3. Hocking, K. S., and Yeo, D. — *Bull. ent. Res.*, **47**: 631-644, 1956.
4. Hocking, K. S., Yeo, D., and Anstey, D. G. — *Bull. ent. Res.*, **45**: 585-603, 1954.
5. Simpson, H. R. — (Unpublished).
6. Hocking, K. S. — (Unpublished) *C.I.R.U. Miscellaneous Report N.° 33*, 1947.
7. Crawford, J. — (Unpublished) *E.A.T.R.O. Report*, 1949.
8. Woodcock, K. E. — *Bur. perm. interafr. Tsetse Tryp.*, N.° 79, 1949.
9. Wilson, S. G. — *Bull. ent. Res.*, **44**: 711-728, 1953.
10. MacLennan, K., Rand, J., and Kirkby, W. W. — *Bull. ent. Res.*, **49**: 123-131, 1958.
11. Whiteside, E. F. — *Bull. ent. Res.*, **40**: 123-134, 1949.
12 Burnett, G. F. — *Bull. ent. Res.*, **45**: 411-421, 1954.

# COMPLEXO POLISSACARÍDO-POLIPEPTIDO DE *TRYPANOSOMA CRUZI* [1]

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*Trypanosoma cruzi* cultivado em meio difásico de Chang com ligeiras modificações foi extraído com hidrato de cloral 33 % a 70°C e precipitado com álcool. Após reprecipitação obtém-se uma fracção bruta de polissacarido. Esta fracção, analisada electroforèticamente no aparelho Tiselius-Spinco, resolve-se em componentes principais de polissacarido e ácido nucleico. O primeiro foi isolado por electoforeses sucessivas (tampão de cacodilato de sódio, força iónica 0,1 e pH = 7,0) e novamente purificado.

A amostra pura contém: N = 4,95 %; P = 0,62 %; carboidrato (como glicose) = 42,0 %. Não é dialisável.

Os componentes do polissacarido, após hidrólise, foram determinados quantitativamente por cromatografia, apresentando as seguintes razões molares: glicose : glicosamina : xilose : manose : galactose = = 1 : 2 : 4 : 5 : 9.

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[1] Trabalho realizado com o auxílio da Rockefeller Foundation e do Conselho Nacional de Pesquisas.

[2] Instituto de Higiene de Tóquio. Bolsista do Conselho Nacional de Pesquisas.

A cromatografia em papel identificou no hidrolisado ácido 11 ácidos aminados.

Esta amostra é considerada um complexo polissacarido-polipeptido; é dotada de actividade imunológica contra soro de paciente de moléstia de Chagas, demonstrada pela técnica ágar-soro de Oudin.

# PROFILAXIA DA DOENÇA DE CHAGAS

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RESUMO

Na falta de recurso terapêutico eficaz, e não existindo processo imunizante, concentra-se a profilaxia da doença de Chagas no combate ao insecto transmissor.

O emprego de insecticidas de acção tóxica residual em expurgos domiciliares permitiu o desenvolvimento de campanhas profilácticas de massa, cujos resultados têm sido bastante animadores.

A profilaxia da doença de Chagas no Brasil, com excepção do estado de São Paulo, é realizada pelo Departamento Nacional de Endemias Rurais, do Ministério da Saúde.

Essas actividades orientam-se segundo os seguintes objectivos:

1) Conhecimento da distribuição dos triatomíneos transmissores;

2) Estudo do grau de prevalência da doença e da sua importância como factor determinante de cardiopatias;

3) Luta contra os transmissores.

O insecticida mais aconselhado é o *hexaclorobenzeno* (B. H. C.), usado sob a forma de pó molhável, na dose de 500 mg de isómero gama por metro quadrado de superfície.

A nossa experiência permite concluir que uma aplicação conveniente desse insecticida é suficiente para reduzir a níveis mínimos ou mesmo eliminar a infestação triatomínica.

Até o ano de 1957 foram realizados 1 318 428 expurgos domiciliares, em 65 783 localidades, de 446 municípios, de 5 estados.

Ao lado da luta contra os «barbeiros», por meio de insecticidas de acção tóxica residual, as chamadas *medidas de profilaxia defensiva*

são, na verdade, aquelas capazes de garantir a solução definitiva do problema. A substituição de casas rústicas (cafuas), com paredes de barro, cheias de rachaduras onde se alojam os triatomíneos, por casas de alvenaria, com paredes lisas, seria o recurso ideal, todavia impraticável em grande escala no momento.

Procurando uma solução imediata, está o D. N. E. Ru. promovendo, em suas campanhas profilácticas, o revestimento das paredes das cafuas com argamassa simples, de fácil emprego e baixo custo, usando-se maquinaria simples.

Ao lado das medidas de profilaxia, o autor considera de inestimável valor as actividades de educação sanitária e legislação adequada.

# ERRADICAÇÃO DO *TRIATOMA INFESTANS* [1]

NÍCIA PINTO DIAS

O *Triatoma infestans* é um dos principais vectores sul-americanos da doença de Chagas, sendo o mais importante em vasta região de clima mesotérmico (1) que abrange partes da Argentina, Chile, Uruguai, Paraguai, Bolívia, Peru e Brasil. O combate a este hemíptero constitui, portanto, grave problema médico-sanitário, que, se satisfatòriamente solucionado, beneficiará uma população de vários milhões de habitantes.

Ao correr dos tempos o *T. infestans* tornou-se uma espécie eminentemente doméstica, cujo *habitat* se limita, de modo pràticamente exclusivo, ao domicílio e às suas dependências imediatas. Tal facto, bem como o da sua grande susceptibilidade a insecticidas de acção residual, como o hexaclorocicloexano (HCH ou BHC), verificada desde os primeiros trabalhos sobre o assunto, e outros factos da biologia do insecto, representam circunstâncias favoráveis ao empreendimento de campanhas que tenham por objectivo final sua total eliminação. Não obstante, as campanhas de grande envergadura contra ele realizadas têm sido de molde a proporcionar apenas uma melhoria transitória da situação, sem chegar a livrar do hematófago as áreas trabalhadas, sendo baseadas em tratamentos domiciliários largamente espaçados, de um ou mais anos. Um dos graves inconvenientes de semelhante táctica de luta é constituído pelo risco que as borrifações larga e irregularmente intervaladas oferecem ao hemíptero para a formação de raças resistentes aos insecticidas, a exemplo do que se tem verificado com outros insectos.

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[1] *O Hospital*, Rio de Janeiro, **55** (4): 603-609, 1959.

Perante os Quintos Congressos Internacionais de Medicina Tropical e Paludismo, Dias (2) manifestou a opinião de que «Quanto à forma de lutar contra os triatomas com insecticidas residuais, acreditamos que deva ser desenvolvida metódica e progressivamente, por regiões, fazendo pelo menos dois expurgos por ano, até que os domicílios fiquem livres desses insectos, o que provàvelmente ocorreria dentro de um ou dois anos na maioria dos casos». Parece óbvio que, como no caso das pragas em geral, também no caso particular dos triatomas se deve tentar executar uma táctica radical de combate, com a qual se procure sanear total e definitivamente uma região, tanto mais quanto para isto oferecem algumas condições especialmente favoráveis os vectores domiciliários da esquizotripanose.

O Instituto Oswaldo Cruz, na gestão do saudoso director Prof. Henrique Aragão, fez instalar em Bambuí, Minas Gerais, Brasil, um centro destinado ao estudo da profilaxia da doença de Chagas, aí em funcionamento desde o ano de 1943 (3). Verificada desde logo (4) a ineficácia do DDT contra as espécies locais, *Triatoma infestans* e *Panstrongylus megistus*, foi pouco depois comprovada a pronunciada acção letal, contra os mesmos, do BHC (5, 6). Ampliadas no município de Uberaba as investigações, com a colaboração do antigo Serviço Nacional de Malária, foi depressa verificado que «Já se dispõe de técnica de comprovada eficácia para a luta contra os transmissores da doença de Chagas» e que «com apenas um expurgo pode conseguir-se enorme diminuição do número de barbeiros das casas infestadas, conseguindo-se a redução a zero em grande número delas» (7), conclusões estas que foram confirmadas em muitos outros trabalhos posteriores, no Brasil e outros países.

Procurando obter ràpidamente a completa destruição dos criadouros de triatomíneos, Dias (8) experimentou em 68 habitações uma táctica de erradicação baseada em duas aplicações domiciliárias de BHC efectuadas com o espaço de 1 mês aproximadamente. Esta táctica se baseou no facto de que o insecticida não possui acção letal contra os ovos dos triatomíneos e na relativamente rápida queda do efeito residual do BHC nas paredes das cafuas, factos esses que anos antes o haviam levado a preconizar a repetição da borrifação ao cabo de 1 a 2 meses (5, 6).

Obtida a completa eliminação dos barbeiros nas 68 casas tratadas, foi efectuada em todo o município de Bambuí um trabalho visando a

erradicação do *T. infestans* e do *P. megistus*, numa primeira campanha experimental, para a qual contou o Instituto Oswaldo Cruz com a decisiva cooperação de um órgão directamente ligado à Presidência da República, a Comissão do Vale do São Francisco, superiormente dirigida pelo Eng. Assis Scaffa, e do Departamento Nacional de Endemias Rurais, então sob a esclarecida gestão do Dr. Mário Pinotti. Sobre o assunto já foram prestadas informações em diversas publicações (9, 10, 11, 12, 13, 14) e conferências (15).

Na campanha experimental, executada numa área de 2425 km² e de Agosto de 1956 a princípios de Fevereiro de 1957, foram borrifadas cerca de 4690 habitações e verificaram-se as três fases seguintes: trabalhos preliminares, que constaram especialmente do levantamento e numeração dos prédios tratados e propaganda sanitária visando o preparo da população e solicitação da cooperação da mesma; borrifação dos domicílios e anexos; eliminação dos focos residuais de triatomíneos.

A destriatomização foi realizada mediante a aplicação de BHC (pó molhável contendo 10 % a 12 % de isómero gama, em suspensão aquosa a 5 %), por duas vezes, com intervalo de cerca de 30 dias. A segunda borrifação foi feita com o propósito de produzir a eliminação das ninfas nascidas depois da primeira aplicação, que poderiam escapar ao efeito residual da mesma. A aspersão de insecticida não foi feita regularmente na superfície das paredes, tal como se faz com o DDT nas campanhas antimaláricas, mas foi feita especialmente nas gretas e buracos das paredes e em todos os possíveis esconderijos de barbeiros (fig. 1). O município foi dividido em diversas zonas, cujas casas foram tratadas em um mês e retratadas no mês seguinte, até completa cobertura de toda a zona de trabalho. Após a segunda borrifação cada área entrava na terceira fase da campanha, durante a qual foram sendo procurados activamente focos residuais de triatoma, que eram tratados na forma descrita. Até Novembro de 1957 foi gasto o total de 206 494 l da suspensão de pó molhável de BHC, tendo sido consumida uma média de 24 l por expurgo e por domicílio e de 20 l nos tratamentos de focos residuais. Houve omissões na execução das borrifações, especialmente em porões de casas de melhor tipo de construção (térreos ou casas de tejolo) e em dependências do peridomicílio, tais como galinheiros, paióis, etc.

Durante a segunda fase da campanha foi capturado um total de 9453 exemplares de *T. infestans* (7484 após o 1.º expurgo e 1969 após o 2.º expurgo) e de 5191 exemplares de *P. megistus* (2915 após o 1.º expurgo e 2276 após o 2.º expurgo), enquanto até 31 de Março de 1958, durante a fase de vigilância, foram capturados 153 *T. infestans* e 840 *P. megistus*. Releva ponderar que as capturas do 2.º período foram feitas pelos próprios moradores das casas tratadas, sem que pudesse ter sido organizado um serviço regular de capturas por guar-

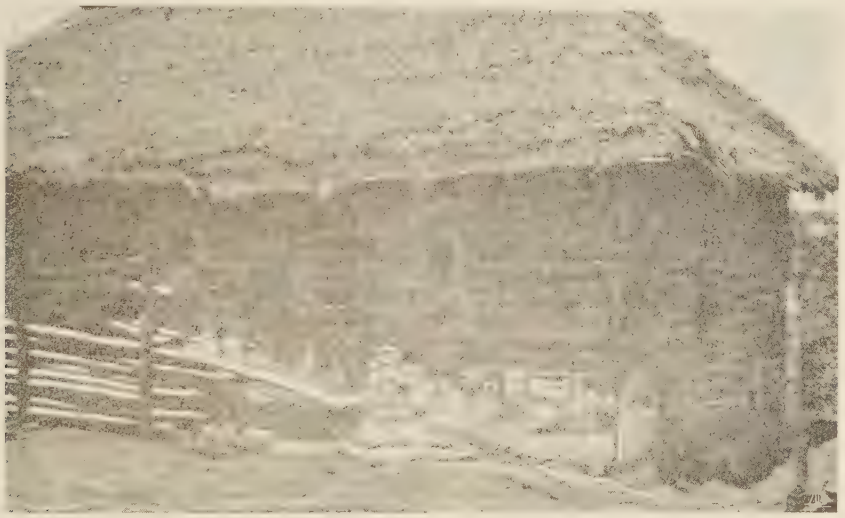

Fig. 1 — Fotografia de cafua de barro tratada com BHC, mostrando a aplicação do insecticida de preferência nas frestas e buracos

das, devendo, pois, ter sido muito maior o número de insectos mortos durante a campanha, assim como o número de domicílios em que foi comprovada a infestação, que atingiu mais de 30 % dos domicílios tratados.

Os focos remanescentes encontrados localizaram-se sobretudo nas habitações de tejolo, tipo «térreo», nos paióis, galinheiros, cobertas e outros abrigos de animais domésticos: enquanto em «térreos» e anexos foram achados 256 focos (83 %), em cafuas e anexos só foram achados 53 (17 %). Não foram aí incluídas 68 habitações em que foram capturados espécimes adultos isolados, sendo 82 *P. megistus*

e 9 *T. infestans*. Assim se distribuíram os focos residuais observados: com respeito aos térreos, 78 % dos focos eram constituídos por *P. megistus*, 15 % por *T. infestans* e 7 % por ambos, e em relação aos ranchos ou cafuas, 82 % eram de focos constituídos por *P. megistus* e 18 % por *T. infestans*. No conjunto, o *P. megistus* estava presente em 79 % dos criadouros, o *T. infestans* em 15,5 % e ambas as espécies em 5,5 %. Durante os trabalhos foi encontrado um exemplar adulto de *Triatoma maculata* e uma ninfa de *Rhodnius* sp., pela primeira vez assinalados no município.

No município de Bambuí, em épocas anteriores aos trabalhos de erradicação, o *T. infestans* era uma espécie muito mais frequente do que o *P. megistus*, representando 94 % dos espécimes colectados em 10 anos (16).

Quanto à incidência das duas espécies nos diferentes criadouros residuais, foram observados os resultados abaixo:

| Locais | Percentagem dos 309 focos constituídos por | |
|---|---|---|
| | *P. megistus* | *T. infestans* |
| Porões . . . . . | 23 | 5 |
| Casas. . . . . | 21 | 5 |
| Galinheiros . . . . . | 10 | 4 |
| Cobertas . . . . . | 10 | 3 |
| Paióis . . . . . | 15 | 2 |
| Pombais . . . . . | — | 0,6 |

Esses focos foram devidos, em sua quase totalidade, a omissões parciais ou totais nas aplicações de insecticida, constituindo 6,6 % do total das habitações expurgadas, e naturalmente seria bem inferior essa percentagem se houvessem sido computadas naquele total todas as dependências dos prédios tratados. Um resultado auspicioso comprovado foi o de que os criadouros clássicos de triatomíneos, as cafuas, que constituíram objecto de minucioso tratamento por parte das *équipes* de expurgo, foram libertados desses criadouros na proporção de 99 % em seguida aos dois expurgos efectuados.

Os focos remanescentes localizados foram tratados da mesma forma que os anteriores e erradicados, sendo actualmente raríssimo

encontrarem-se barbeiros na região, já tendo sido eliminados alguns focos novos em residências ocupadas ou construídas por moradores procedentes dos municípios circunjacentes, todos eles ainda densamente infestados por esses triatomíneos. Há cerca de 2 anos não são registados casos agudos de doença de Chagas em Bambuí, enquanto alguns têm sido diagnosticados no Posto do Instituto Oswaldo Cruz, procedentes dos municípios vizinhos.

Focos silvestres de *T. infestans* e *P. megistus* nunca foram encontrados em Bambuí.

Em face do exposto, fazemos nossas as seguintes palavras (17):

> «Acreditamos ter ficado demonstrado que as bases adoptadas na presente campanha são bem fundamentadas e que se pode, portanto, afirmar, como conclusão mais geral dela decorrente, que:
>
> *Obtém-se a destruição total dos barbeiros sempre que seus criadouros forem convenientemente tratados com BHC por duas vezes, ao intervalo de um mês aproximadamente.*
>
> Consequentemente, se todos os criadouros de uma região forem submetidos a esse tratamento, o resultado deverá ser a eliminação das espécies visadas dessa região. As campanhas de erradicação serão coroadas de tanto maior êxito quanto mais rigorosamente forem realizadas. Nelas haverá sempre a possibilidade de falhas de execução, que não há que confundir com falhas de método. Haverá falência deste quando focos convenientemente tratados não forem eliminados.
>
> O presente esquema de erradicação é de tal maneira simples que dificilmente poderá ser superado, a não ser que se venha a encontrar um insecticida capaz de promover a eliminação total com uma única aplicação.
>
> Não tendo o BHC, que é ainda geralmente considerado o melhor triatomicida, essa capacidade, conforme o tem demonstrado a experiência, tem-se que recorrer ao estabelecimento de um ciclo de aplicações, sobre o qual os autores nunca chegaram a um acordo. Longe de ser um detalhe empiricamente fixável, é este o ponto fundamental da questão. Qual o intervalo óptimo entre as aplicações? Qual o número destas necessário para que se atinja o fim colimado, que é a protecção eficaz das populações

contra os nefastos insectos responsáveis pela transmissão da grave endemia?

Não devemos correr o risco de perder esta poderosa arma contra os barbeiros, dando ao insecto *chance* para adquirir-lhe resistência pelo seu manejo inadequado. Tenhamos presente o que está acontecendo com o DDT e meditemos no que disse Hess (18):

> «We need also to return to basic studies on the biology and habits of insects. This type of research has been sorely neglected during the DDT era, when many seemed to believe that the only knowledge one needed in order to control insects was the operational procedures for a DDT residual spray program.»

Uma vez que é reconhecida a necessidade de «ciclos de aplicação» do BHC, não parece difícil admitir que o ciclo mínimo possível, e por conseguinte o mais económico, é o de duas aplicações; e se o intervalo biològicamente fixado para as mesmas é mínimo, como pretender que o método proposto seja excesssivamente dispendioso ou excessivamente demorado? Ao contrário, ele é o mais rápido, radical e económico de quantos têm sido empregados, mormente os que prevêem as borrifações longamente intervaladas e por tempo indeterminado.»

Tudo leva a crer haverá de produzir bons resultados o esquema para a campanha de erradicação de triatomíneos domiciliários proposto por Dias (19), aplicável a grandes regiões:

> «1. Realização, a mais rigorosa e integral possível, das duas borrifações indicadas nos domicílios e anexos, com o que fica pràticamente interrompida a transmissão da moléstia, como se observou em Bambuí, deixando a endemia de constituir problema de saúde pública na região, abstracção feita, naturalmente, dos casos já existentes, que continuarão na esperança de uma terapêutica específica;
>
> 2. A intervalos anuais, ou mesmo bianuais, a região será percorrida por turmas de inspecção, que localizarão os focos

remanescentes ou de reinfestação e os eliminarão como precedentemente. O custo da campanha, que será elevado no primeiro ano, baixará notàvelmente nos anos seguintes, até que o objectivo da erradicação possa ser considerado atingido. Esta táctica resolve bem os eventuais casos de reinfestação à distância e a partir de focos remanescentes nas adjacências do domicílio ou mesmo silvestres e se aplica, aparentemente, a todos os grandes transmissores da doença de Chagas.»

Podemos concluir desejando que não tarde em ser posto em prática o conceito de erradicação dos grandes transmissores da esquizotripanose, como aconteceu com o da erradicação da malária, tal como nos é relatado por Williams, Jr. (20):

«The concept of the eradication of malaria was born in the United States, but its growth was slow. It had to overcome the dictum that, while malaria could be controlled, eradication should not be attempted.

The budding malariologists had this point of view persistently drilled into them by their elders, who held that it was almost presumptuous to attempt eradication. Control the disease through antilarval works, yes; eliminate it, no — fantastic!»

## BIBLIOGRAFIA


1. Aragão, M., e Dias, E. — «Aspectos climáticos da doença de Chagas», *Revista Brasil. de Malariol. e D. Tropicais*, vol. VIII, 4: 633-641, 1956.
2. Dias, E. — «Comentario al trabajo del doctor C. Romaña (Enfermedad de Chagas)», *Comptes Rendus des Vmes Congrès Internationaux de Médecine Tropicale et du Paludisme*, Istanbul, 644-645, 1954.
3. Dias, E. — «O Centro de Estudos e Profilaxia de Moléstia de Chagas em Bambuí, estado de Minas Gerais. Notícia histórica em homenagem ao Professor Henrique Aragão», *Memórias do Instituto Oswaldo Cruz*, **54** (1): 309-357, 1956.
4. Dias, E. — *Um ensaio de profilaxia de moléstia de Chagas*. Imprensa Nacional, 116 pp., 81 figs., 1945.
5. Dias, E., e Pellegrino, J. — «Alguns ensaios com o gamexano no combate aos transmissores da doença de Chagas», *Brasil Médico*, **62**: 185-191, 1948.
6. Dias, E. — «*Contrôle* das doenças transmitidas pelos triatomas», *Boletim da*

*Oficina Sanitária Panamericana*, vol. XXVII, **12**: 1160-1164, 6.ª Conferência Panamericana de Directores Nacionais de Saúde, México, Outubro, publ. n.º 243 da Of. Sanit. Panamericana, pp. 123-127, 1948.

7. DIAS, E., PINTO, O. S., PELLEGRINO, J., e CASTRO, A. — «Ensaio experimental de luta contra os triatomíneos por meio de insecticidas de acção residual», *Rev. Brasil. Malariologia e D. Tropicais*, **4**: 62-64, 1952.
8. DIAS, E. — «Plano de erradicação dos triatomíneos domiciliários no município de Bambuí, Minas Gerais», *Jornal do Comércio*, Rio de Janeiro, 10 de Agosto de 1956.
9. DIAS, E. — «Profilaxia da doença de Chagas», *O Hospital*, vol. LI, **3**: 285-298, 1957; *Jornal do Comércio*, Rio de Janeiro, 2 de Dezembro de 1956.
10. DIAS, E. — «Sugestões preliminares para um plano de erradicação dos transmissores domiciliários da doença de Chagas no estado de São Paulo», *Revista Médica do Sul de Minas*, vol. III, **3**: 104-113, 1957.
11. DIAS, E. — «Apresentado plano para erradicação da moléstia de Chagas no estado de São Paulo», *O Estado de São Paulo*, 29 de Setembro de 1957.
12. DIAS, E. — *Epidemiologia y profilaxis de la enfermedad de Chagas*. Resumo mimeog. de conferência pronunciada na Academia Nacional de Medicina, Buenos Aires, 3 de Outubro de 1957.
13. DIAS, E. — «Aspectos sanitários da luta antichagásica no Brasil», Primeiras Jornadas Venezuelanas de Cardiologia, Caracas, 18 a 22 de Novembro de 1957, *O Hospital*, **54** (4): 471-482, 1958.
14. DIAS, E., e PINTO DIAS, N. — «Ausröttung von Raubwanzen in Bambui, Brasilien», *Zeitschr. f. Tropenmedizin u. Parasit.* **9** (4): 291-293, 1958.
15. DIAS, E. — Conferências e palestras: Buenos Aires (Pavilhão Inchauspe do Hospital Ramos Mejia; Instituto Nacional de Microbiologia; Cátedra de Parasitologia e Microbiologia da Faculdade de Medicina; Oficina Sanitária Panamericana); Montevideu (Ministério da Saúde e Instituto de Higiene); Venezuela (Maracay, Divisão de Malariologia; San Juan de los Morros, Serviço do Dr. J. F. Torrealba; Caracas, Ministério da Saúde); Bogotá (Ministério da Saúde); Lima (Instituto Nacional de Higiene e Oficina Sanitária Panamericana); Santiago (Departamento de Parasitologia da Universidade do Chile): Outubro, Novembro e Dezembro de 1957.
16. DIAS, E. — «Variações mensais da incidência das formas evolutivas do *Triatoma infestans* e do *Panstrongylus megistus* no município de Bambuí, estado de Minas Gerais», *Memórias do Instituto Oswaldo Cruz*, **53**: 457-472, 1955.
17. DIAS, E. — Relatório da Campanha apresentado aos directores do Instituto Oswaldo Cruz, Departamento Nacional de Endemias Rurais e Comissão do Vale do São Francico, 1957.
18. HESS, A. D. — *Am. Jl. Trop. Med. Hyg.*, **3**: 371-388, 1952.
19. DIAS, E. — Ofício de 17-4-1958 ao Sr. Director do Departamento Nacional de Endemias Rurais, Dr. Mário Pinotti, sobre erradicação de triatomas, 1958.
20. WILLIAMS, JR., L. L. — «Malaria eradication — Growth of the concept and its application», *Am. Jl. Trop. Med. Hyg.*, vol. VII, **3**: 259-267, 1958.

# ANTIBODIES PRODUCED IN THE RAT DURING INFECTION WITH *TRYPANOSOMA LEWISI*

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Inhibition of reproduction of *T. lewisi* early in the infection of rats, which was first noted by Rabinowitsch and Kempner (1899), was shown to be the result of an antibody (Taliaferro, 1924) called *ablastin* (Taliaferro, 1932). In addition to ablastin, Coventry (1930) found an early trypanocidal antibody and Taliaferro (1938) reported that a typical lysin terminates the infection. These antibodies give infections of *T. lewisi* a unique character (Taliaferro, 1948). Other interpretations of the activities of these antibodies have been advanced. Thus, Regendanz and Kikuth (1927) associated the disappearance of trypanosomes from the blood with nonspecific phagocytosis. Barnes (1951) emphasized the role of agglutinating antibody in the disposal of the parasites. Most recently, Thillet and Chandler (1957) concluded that some of the effects ascribed to trypanocidal antibodies are caused by ablastin acting as an agglutinin.

The aims of the present work were to ascertain the physicochemical properties of ablastin and the two trypanocidal antibodies and especially to study the effects of ablastin on nucleic acid and protein synthesis of the trypanosomes.

## MATERIALS AND METHODS

The terms early and terminal trypanocidal antibodies are used, respectively, for the passively transferable properties found in serums of rats (1) taken from about 10-day infections and (2) after the termination of infections or superinfections.

Each trypanocidal antibody was tested *in vivo* by administering graded doses of appropriate antiserum intraperitoneally to rats infected with trypanosomes and making daily observations of the blood for 7 to 10 days to ascertain whether the trypanosomes disappeared. Fresh mounts and Giemsa-stained smears were made.

Ablastic activity was tested *in vivo* as follows. Graded doses of ablastin-containing serum from which the trypanocidal antibodies had been removed by absorption were injected intraperitoneally into rats infected with trypanosomes. Daily observations of the blood in Giemsa-stained smears were then made for 10 days to ascertain whether the trypanosomes ceased dividing. Ablastin was considered not to be present if the trypanosomes divided. For convenience, the non-dividing trypanosomes are called adults or inhibited parasites. Trypanosome infections were initiated with either intraperitoneal or intravenous injections of 200 to 400 $\times$ $10^6$ dividing or adult trypanosomes per 100 g rat. On the 3rd day of the infection there were about 10,000 growing and dividing parasites per cubic millimiter of blood, of which 20 to 45 % were small, dividing or large and on the 8th to 11th day there were about 70,000 parasites per cubic millimiter of blood, of which all were uniform in size and adult in appearance.

For the study of protein and nucleic acid synthesis, infected rats received a single intraperitoneal injection of 0.01 mc per 100 g rat of $S^{35}$-labeled yeast hydrolysate or of adenine-8-$C^{14}$ sulfate. After variable intervals, trypanosomes were collected from the rats by the method described by Moulder (1948). They were then washed and mixed with trichloroacetic acid (= TCA) to make a final concentration of 10 %. The resulting precipitate was washed twice in 5 % TCA containing unlabeled cysteine and methionine or adenine sulfate to allow for exchange reactions. The TCA precipitate was finally dissolved in 0.04 *N* NaOH and plated. Radioactive counts are expressed

per $10^6$ trypanosomes (whether they are dividing or adult, since samples of each measured the same with a planimeter).

## PHYSICO-CHEMICAL CHARACTERIZATION

No separation of the antibodies could be made by starch block electrophoresis using a modification of the method of Kunkel and Slater (1951). All three antibodies showed similar mobilities and localized between the $\beta$ and $\gamma$ globulins.

The approximate sedimentation constants in Svedbergs for the three antibodies were determined in a Spinco model E analytical ultracentrifuge. The terminal lysin was a large molecule (16 S) probably approximating a molecular weight of 1,000,000, whereas the early trypanocidal antibody and ablastin were smaller (6 S), with an approximate molecular weight of 150,000. It is thus evident that only the terminal lysin can be separated from the other two antibodies by ultracentrifugation. Ablastin can, however, be separated from the two trypanocidal antibodies by absorption with living trypanosomes. Thus, trypanosomes absorb the trypanocidal antibodies and leave ablastin in the serum, as shown by Taliaferro (1932). The lack of absorbability of ablastin is apparently due to a masking or an inaccessibility of the antigen and/or a high dissociation rate of the antigen-antibody complex.

## NUCLEIC ACID SYNTHESIS

In preliminary experiments, we found that guanine-8-$C^{14}$ was not incorporated into *T. lewisi* in appreciable amounts, whereas adenine-8-$C^{14}$ sulfate was readily incorporated into dividing forms.

Subsequent work involved the following. Adenine-8-$C^{14}$ was administered (1) to 8 rats on the 3rd day of the infection and (2) to 11 rats on the 10th day. Incorporation of $C^{14}$ into the TCA precipitate of dividing and growing trypanosomes from the first group showed a mean specific radioactivity of $264 \pm 45$ counts per minute per $10^6$ trypanosomes, whereas the adult trypanosomes from the second group showed only $8.5 \pm 3$ counts per minute per $10^6$ trypanosomes.

This latter count was essentially negative. These findings indicate that all nucleic acid synthesis is virtually inhibited in trypanosomes which have had their reproduction and growth inhibited by ablastin.

## PROTEIN SYNTHESIS

$S^{35}$-labeled amino acids in the form of a yeast hydrolysate were injected (1) into 46 rats containing adult trypanosomes and (2) into 47 rats containing growing and dividing trypanosomes. Incorporation of $S^{35}$ into the TCA precipitate of the adult trypanosomes rose to a count of $19 \pm 3$ per minute per $10^6$ parasites at 1.5 hours. Very little decrease from this level occurred during the ensuing 22 hours. In marked contrast, the dividing parasites acquired about three times the amount of radioactivity, viz. $62 \pm 9$ counts per minute per $10^6$ parasites at 2 hours. By 24 hours this count had dropped to $19 \pm 3$, i.e., the value for adult parasites. These findings leave little doubt that the dividing parasites incorporate more amino acids into protein than do the inhibited forms. Our work so far does not indicate how much of the $S^{35}$ uptake, especially in the adult parasites, is nonspecific, i.e., represents an exchange reaction with the amino acids of existing proteins or a nonspecific adsorption of nonantibody proteins or how much protein is degraded by the parasites. These unsolved problems do not invalidate the conclusion that the dividing parasites synthesize much more protein than do the inhibited adult parasites.

## DISCUSSION AND SUMMARY

The most striking findings in the present work are as follows: (1) All three of the antibodies studied localize during starch block electrophoresis between the fast moving $\gamma$ globulins and the slow moving $\beta$ globulins. (2) The trypanolysin terminating the infection is a large molecule with an approximate molecular weight of 1,000,000, whereas the early trypanocidal antibody and ablastin are small molecules with an approximate molecular weight of 150,000. (3) Ablastin practically inhibits the synthesis of nucleic acid, as shown by the incorporation of $C^{14}$ into the trichloroacetic acid precipitate of dividing but not of adult trypanosomes when adenine-8-$C^{14}$ is administered to the host. (4) In addition, ablastin reduces protein synthesis, as shown by the greater incorporation of $S^{35}$ into the trichloroacetic acid precipitate of dividing trypanosomes, as compared to adult

trypanosomes when $S^{35}$-labeled amino acids is administered to the host. The reduction in protein synthesis by the trypanosomes inhibited by ablastin may be greater than indicated by the $S^{35}$ uptake, since some of the uptake may represent adsorption of $S^{35}$-labeled serum protein on the trypanosomes. This work has been published in final form by D'Alesandro (1959) and by Taliaferro and Pizzi (1960).

Our results on nucleic acid and protein synthesis in a sense parallel those on glucose metabolism reported by Moulder (1948). Moulder showed that ablastin shifts the glucose metabolism from a state of active assimilation to one of maintenance.

## BIBLIOGRAPHY


Barnes, E. A. — *Iowa State Coll. J.*, **26**: 1-17, 1951.

Coventry, F. A. — *Amer. J. Hyg.*, **12**: 366-380, 1930.

D'Alesandro, P. A. — *J. Infect. Dis.*, **105**: 76, 1959.

Kunkel, H. G., and Slater, R. J. — *Proc. Soc. Exper. Biol. and Med.*, **80**: 42-44, 1951.

Moulder, J. W. — *J. Infect. Dis.*, **83**: 42-49, 1948.

Rabinowitsch, L., and Kempner, W. — *Ztsch. f. Hyg. u. Infektionskr.*, **30**: 251-294, 1899.

Regandanz, P., and Kikuth, W. — *Centralbl. f. Bakt. Orig.*, **103**: 271-279, 1927.

Taliaferro, W. H. — *J. Exper. Med.*, **39**: 171-190, 1924.

Taliaferro, W. H. — *Amer. J. Hyg.*, **16**: 32-84, 1932.

Taliaferro, W. H. — *J. Immunol.*, **35**: 303-328, 1938.

Taliaferro, W. H. — *Bact. Rev.*, **12**: 1-17, 1948.

Taliaferro, W. H., and Pizzi, T. — *Proc. Nat. Acad. Sc.*, **46**: 733, 1960.

Thillet, Jr., C. J., and Chandler, A. A. — *Science*, **125**: 346-347, 1957.

## GENERAL DISCUSSION

Dr. Emmanuel Dias (Brasil) — Consideramos feliz a circunstância de achar-se ao nosso lado, como Chairman, o Dr. Soper, quando lemos o nosso relatório, escrito de tão longe e com a intenção de fazê-lo chegar ao seu conhecimento. Que não se veja nas nossas palavras, ditas e por dizer, nada de pessoal contra o director da Repartição Sanitária Panamericana, mesmo porque o que há de pessoal lhe é merecidamente favorável, por lhe reconhecermos o justo título de *erradicador* pioneiro.

O facto inconteste é que, a despeito do que a Repartição já fez em relação à doença de Chagas, ela ainda está pràticamente alheia ao problema, não obstante os diversos apelos que lhe têm sido feitos em congressos, reuniões e trabalhos científicos.

Admito que não cheguei a perceber a razão deste facto, como era de se esperar dos comentários do ilustre director da Repartição Sanitária Panamericana.

A alusão que fez ao nome do descobridor da doença não tem razão de ser, pois o Dr. Carlos Chagas não viveu o bastante para ver o quadro continental da doença, nem a seu tempo havia a mínima possibilidade de lutar contra a endemia.

Disse o Dr. Soper que não é suficiente mostrar a importância de um problema, nem conhecer-lhe as soluções: o fundamental é saber «vender a ideia» *(sell the idea)*.

Seria muito importante que ele nos esclarecesse melhor sobre o particular.

Por exemplo, *quem* deve saber «vender a ideia»? Até hoje ninguém soube fazê-lo?

Quem deverá comprá-la? Que é preciso fazer para que as maiores autoridades sanitárias do mundo, com seu alto discernimento, comecem a se preocupar com a doença de Chagas?

Não estão ainda convencidas de sua importância? Se estão e se são elas que devem *comprar* a ideia, ainda não se acham dispostas a fazê-lo? Se não são, não poderão ajudar a vendê-la, ou ensinar a fazê-lo?

Creio que são estes os termos em que deveremos dirigir mais este apelo à Repartição Sanitária Panamericana, representante regional da Organização Mundial da Saúde.

Dr. E. Friedheim (U.S.A.) — On a voulu expliquer la différence observée dans le nombre d'accidents attribués au Mel B par une différence dans la composition chimique du produit.

Je tiens à souligner le fait que les lots de Mel B préparés, apportés et distribués en Afrique étaient chimiquement purs, toujours identiques. C'est un échantillon de ces lots qui a été déposé à Londres comme standard, à la demande de l'O.M.S.

Je me permets de demander aux rapporteurs ce qu'ils pensent de l'association tryparsamide-Mel B et de l'application du Mel B dans le traitement de la première période.

Dr. G. Neujean (Belgique) — *Réponse au Docteur Friedheim:*
1) L'arsobal, à la dose unique de 4 mg/kg, donne en première période le maximum de chance de guérison;

2) L'association de la tryparsamide à l'arsobal ne me paraît absolument pas défendable. À la rigueur, pourrait-on faire précéder l'arsobal d'une dose modérée d'un trypanocide périphérique.

Dr. Robert Dezowitz (U.S.A.) — Dr. Mornet gave the impression, possibly as a result of translation, that all suramin complexes are toxic. This, of course, is not the case; suramin tends to toxicate the basic drug with which it forms a complex. Berenil, for instance, can be given in very large amounts as a complex; unfortunately, however, the complex exhibits little prophylactic activity. I am sure that suramin complexes, in some forms, will be used to give long term prophylaxis against bovine trypanosomiasis.

Dr. Rudolf Fussgänger (Germany) — Le rapport du Docteur Mornet me donne l'occasion de quelques remarques sur le bérénil, notre médicament allemand actif à la fois contre la maladie de la

tsé-tsé, causée par le *Trypanosoma congolense* et *vivax* et par les babésioses.

Une question de grand intérêt est, à mon avis, celle de savoir quelle est la méthode la plus appropriée, dans les conditions de laboratoire, pour trouver un médicament efficace contre les infections à babésies.

Dans notre laboratoire à Francfort, nous expérimentons depuis longtemps déjà avec une souche hautement infectieuse de *Babesia canis* qui rend le chien malade en peu de jours et conduit toujours à la mort comme suite d'une hémoglobinurie grave. L'évolution de la maladie est contrôlée au moyen de frottis de sang.

Au cours de nos recherches, nous avons constaté que le bérénil supprime en quelques heures l'hémoglobinurie. Les parasites disparaissent du sang et les chiens redeviennent sains du point de vue clinique. Autrement dit: aux doses habituelles, les chiens restent dans un état de prémunition. Les essais effectués dans plusieurs autres formes de babésioses ont montré que l'expérimentation sur le chien normal convient parfaitement à la sélection de médicaments permettant d'obtenir de bons résultats cliniques.

Tout le monde est persuadé qu'il serait préférable d'abandonner les recherches de routine sur le chien en faveur de l'essai sur de petits animaux (rats et souris). En effet, plusieurs chercheurs ont utilisé, dans leur programme de recherches, la découverte faite par van Den Berghe et autres de la transmissibilité de *Babesia rodhaini* aux souris blanches qui en meurent. Il y a deux ans, Ryley publia, en Angleterre, une étude traitant des bons résultats qu'il avait obtenus dans les babésioses avec le Produit 10073, un nouveau dérivé phénylique de l'antrycide. Il avait expérimenté cette substance sur *Babesia rodhaini* de la souris, sur *Babesia bovis* du veau splénectomisé, ainsi que sur *Babesia canis* du chien splénectomisé.

Le tableau que voici illustre l'efficacité de différentes substances contre *Babesia rodhaini* de la souris. La prochaine colonne reproduit l'index résultant de la dose minimum efficace par comparaison à la dose minimum tolérée conformément au tableau de Ryley. Selon ces données, le Produit 10073 semble avoir, de toutes les substances examinées, l'index le plus favorable. Sur l'animal splénectomisé aussi, Ryley constata une supériorité d'efficacité du Produit 10073 par rapport au bérénil.

Opinant que l'infection de l'animal splénectomisé n'est pas naturelle, nous avons expérimenté les mêmes substances sur le chien normal infesté par notre souche de *Babesia canis*. Le résultat surprenant de ces essais est reproduit à droite de ce tableau.

| | *Babesia rodhaini* | | *Babesia canis* | |
|---|---|---|---|---|
| | Mouse | | Dog | |
| | Minimum effective dose (mg/kg) | de : dt [1] | Minimum effective dose (mg/kg) | de : dt [1] |
| Acapron . . . . . | 1,5 sc. | 1:10 | 0,5 im. | 1:8 |
| Antrycide . . . . . | 15,0 sc. | 1:5 | 2,5 im. | 1:5 |
| Bérénil . . . . . | 15,0 sc. | 1:2,5 | 0,5 im. | 1:30 |
| 10073 . . . . . | 0,75 sc. | 1:100 | 2,0 im. | 1:12,5 |

[1] $\text{de} : \text{dt} = \frac{\text{minimum effective dose}}{\text{maximum tolerated dose}}$

Alors que l'acapron et l'antrycide révèlent sur les deux modèles une efficacité analogue et un index comparable, le bérénil et le 10073 ont changé réciproquement de position. Chez le chien normal, le bérénil a cette fois-ci un index beaucoup plus favorable que le 10073. Ce résultat concorde avec la haute efficacité dont le bérénil a fait preuve dans diverses régions du globe. De tels médicaments n'étant jamais administrés à des animaux splénectomisés, mais uniquement à des animaux normaux, il m'est incompréhensible que l'auteur ait effectué ses recherches sur de telles infections-modèle s'écartant des conditions naturelles.

Nos résultats chez le chien normal montrent, sans nul doute, que ni l'animal splénectomisé, ni la souris infestée par *Babesia rodhaini* ne conviennent comme modèles à la sélection de nouvelles drogues et à la détermination des préparations les plus efficaces pour le traitement des babésioses dans les conditions de la pratique.

Je saisis volontiers cette occasion pour m'acquitter d'une dette de reconnaissance envers tous les professionnels de renom, experts en matière de babésioses et de maladies de la tsé-tsé, qui, grâce à leur précieux appui, nous ont permis de mener à bien l'expérimentation du bérénil dans les conditions naturelles en Afrique, Proche et Extrême Orient. En les remerciant tous, j'ai le plaisir de m'adresser spécialement à vos excellents spécialistes des colonies portugaises.

Dr. Durval Lucena (Brasil) — *To Dr. Packchanian:* I should ask for Dr. Packchanian to put up to date his epidemiological chart of distribution of Chagas' disease in Brazil.

Northeastern states, where live more than 11 million people, have been included in the endemic area after entomological and serological surveys made and clinical cases reported in the five states included in that physiographical region.

## RÉUNION EXTRAORDINAIRE SUR LES TRYPANOSOMIASES

Le vendredi 12 septembre, à 10 heures 30, il y a eu une réunion hors programme consacrée à la présentation de communications libres sur les *trypanosomiases.*

# TERMINAL RESPIRATION IN MAMMALIAN TRYPANOSOMES

J. D. FULTON
and
D. F. SPOONER

This paper describes a survey of terminal respiration in mammalian trypanosomes, and an opportunity has been presented to contribute to comparative biochemistry. Von Brand (1952) has correlated the cyanide sensitivity of trypanosome respiration with Hoare's (1957) classifications of these parasites (Table 1), and it is of obvious phylogenetic and chemotherapeutic importance to know if cyanide sensitivity is synonymous with the presence of a functional cytochrome system.

The strains of trypanosomes employed were maintained by syringe passage in laboratory rats with the exception of *T. cruzi,* which was maintained in mice. Sufficient material was usually provided for a respiratory experiment by 3 heavily infected rats. In most cases trypanosomes were isolated free from other cells by differential centrifugation without difficulty by methods previously described (Fulton and Spooner, 1956).

TABLE 1 — *Classification and characters of trypanosomes studied, based on Hoare (1957)*

| | *Lewisi* group | | *Vivax* group | *Congolense* group | *Brucei* group | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Brucei* subgroup | | *Evansi* subgroup | | |
| | *T. lewisi* | *T. cruzi* | *T. vivax* | *T. congolense* | *T. gambiense* | *T. rodhesiense* | *T. evansi* | *T. equinum* | *T. equiperdum* |
| Mamalian hosts . . | Rats | Man dog Cat Armadillo Opossum | Bovines Sheep Goat Antelope | Bovines Equines Pig sheep Goat dog | Man | Man Antelope | Bovines Equines Camel Dog | Equines | Equines |
| Insect host or vector | Fleas | Triatomid bugs | Tsetse flies | Tsetse flies | Tsetse flies | Tsetse flies | Tabanid flies | Tabanid flies | None |
| Distribution . . . . | Cosmopolitan | America | Tropical Africa | Tropical Africa | Tropical Africa | Tropical East Africa | Cosmopolitan | S. America | Europe Africa Asia |

Conventional Warburg apparatus was used for measuring oxygen uptake, at 25° or 38°, of suspensions of the organisms in 10 % horse serum in phosphate buffered saline containing 0.2 % glucose. The effect of $0.46 \times 10^{-3}$M cyanide on the respiration of the trypanosomes is shown in Table 2. Only the blood forms of *T. lewisi* and *T. cruzi* and the culture forms of trypanosomes tested were notably sensitive to cyanide, but when *T. congolense* and the «M» strain of *T. rhodesiense* were accompanied by white cells, sensitivity to this agent was observed. When white cells were removed from *T. congolense* preparations by filtration through sintered glass, as described by Fulton and Spooner (1957), no significant inhibition was caused by cyanide. In some instances the respiration of African trypanosomes, notably *T. vivax*, was stimulated by cyanide.

Cyanide is not a specific inhibitor of the cytochrome system and thus sensitivity of trypanosome respiration to it does not necessarily indicate the presence of a cytochrome system. More specific methods have been applied to these organisms to only a limited degree by other workers (Baernstein and Tobie, 1951; Ryley, 1951). By the use of light reversal of carbon monoxide and spectroscopic examinations, however, we have confirmed that an iron catalysed respiratory system does operate in these cyanide sensitive trypanosomes.

*T. lewisi* was the only blood stream form significantly inhibited by carbon monoxide in the dark, but lack of material prevented examination of the bloodstream form of *T. cruzi*. The other species were either not inhibited or were slightly stimulated. In contrast the 3 culture forms examined were all significantly inhibited (Table 3).

When oxygen was measured in alternate periods of light and dark, some reversal by light of all inhibitions occurred, although this was never complete under our conditions. In contrast to Baernstein (1953), we have observed that respiration of the culture form of *T. cruzi* is partly inhibited by carbon monoxide in the dark, but no clear reversal of inhibition by light was demonstrated.

Spectroscopic examination of trypanosome suspensions for the presence of cytochrome bands was made in a Leitz apparatus at room temperature and after cooling to the temperature of liquid nitrogen in 50 % glycerol (Keilin and Hartree, 1949). Those trypanosomes whose respiration was inhibited by carbon monoxide in the dark were seen to contain pigments with similar absorption bands to cytochromes

TABLE 2 — *Percentage inhibition of respiration by cyanide*

| | *Lewisi* group | | *Vivax* group | *Congolense* group | *Brucei* group | | | | | | | | | Culture forms | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *T. lewisi* | *T. cruzi* | *T. vivax* | *T. congolense* | *T. rodhesiense* | | | *T. evansi* | | | *T. equinum* | *T. equiperdum* | | *S. oncopelli* | *T. gambiense* | *T. cruzi* |
| | | | | | L | M | J | NS | E | SAK | ZU | OE | FE | | | |
| $0.46 \times 10^{-3}$M. . | — | — | — | 0.5 | — | — | — | — | — | — | — | — | — | — | — | — |
| Cyanide . . . . . | (85) | (71) | (+ 21) | (24 *) | (9) | (26) | (8) | (+6.5) | (+1) | (+6) | (8) | (+6) | (+12) | (82) | (72) | (72) |

+ denotes stimulation of $O_2$ uptake.
* denotes white cells present.

TABLE 3 — *Effect of carbon monoxide on respiration of trypanosomes (percentage inhibition in the dark)*

| *Lewisi* group | *Vivax* group | *Congolense* group | *Brucei* group | | | | | | | | | Culture forms | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *T. lewisi* | *T. vivax* | *T. congolense* | *T. rhodesiense* | | | *T. evansi* | | | *T. equinum* | *T. equiperdum* | | *S. oncopelli* | *T. gambiense* | *T. cruzi* |
| | | | L | J | M | C | H | SAK | | FE | OE | | | |
| (39) | (+ 9) | (+ 15) | (8) | (1) | (1) | (3) | (+3) | (5) | (2) | (5) | (0) | (62) | (63) | (30) |

+ denotes stimulation of $O_2$ uptake.

«a» and «b». *S. oncopelti* and *T. lewisi* appear also to contain cytochrome «c», but bands characteristic of this pigment are absent from *T. cruzi* and *T. gambiense* culture forms. Ryley (1951, 1955, 1956) and Baernstein (1953) have also reported similar bands in *S. oncopelti, T. lewisi* and *T. cruzi,* but this is the first demonstration of cytochromes in the culture forms of an African trypanosome. It seems that the system is similar in culture forms of African and South American trypanosomes. No bands were seen in suspensions of the other trypanosomes (the blood stream form of *T. cruzi* was not examined). Attempts were made to assay cytochrome oxidase in the trypanosomes. They were disrupted in a Hughes press at — 20°C and cytochrome oxidase assays carried out manometrically and spectrophotometrically. Those trypanosomes which gave positive evidence for a cytochrome system gave negative, or very low values, in the manometric assay of cytochrome oxidase. In no case was the value higher in the presence of added cytochrome «c», as would be expected if cytochrome oxidase was functioning. The more sensitive and specific spectrophotometric assay also gave extremely low oxidase activities, compared with mammalian and other tissues, when used with homogenates of cytochrome-containing trypanosomes. This is puzzling in view of the relatively high $QO_2$ of these parasites. Seaman (1956), working with *S. oncopelti* and *T. cruzi,* has also reported values of a low order. He has attributed this partly to a powerful succinic dehydrogenase in the parasites. This enzyme, by utilizing the small amounts of succinate in the homogenates, reduces the cytochrome «c» in the cuvette as quickly as it is oxidized, thus masking the oxidase. Another possible explanation of these low cytochrome oxidase values may be that, like bacterial cytochrome oxidases (Smith, 1954), trypanosome cytochrome oxidases can only oxidize mammalian cytochrome «c» sluggishly or perhaps not at all. It is also possible, as Seaman (1956) suggests, that the enzyme is «inactivated during the preparations of the homogenate». However, the Hughes press gave better results than the other methods of disintegration which were tested.

At least two distinct types of respiratory enzyme systems are thus found among the *Trypanosomidae* examined, since a marked difference in cyanide sensitivity is apparent. This difference exists not only between bloodstream forms of different species, but also

between insect and bloodstream forms of the same species. However, our observations support Hoare's classification to only a limited degree. The intermediary position with regard to cyanide sensitivity that von Brand claims for *T. congolense* (von Brand and Tobie, 1948; von Brand *et al.*, 1950), which was also reported by Ryley (1956), and the sensitivity of *T. vivax* to cyanide claimed by Desowtiz (1956) were not evident in our experiments. Ryley (1956) has likewise failed to observe this sensitivity of *T. vivax* to cyanide. Both these species cause marked leucocytosis in experimental infections in the rodent and it seems likely that reports of the cyanide sensitivity of these two species is due to contamination of trypanosome suspensions by white cells. It is known that the respiration of leucocytes is sensitive to cyanide (McKinney *et al.*, 1952).

It is not clear whether the transition from a cytochrome to a non-cytochrome pathway during the life cycle of the African trypanosomes occurs in the mammalian host after initiation of infection or in the insect at the metacyclic stage of development. A study of the biochemical stimulus and mechanisms involved in this transition would be most interesting, but, unfortunately, development of the insect forms *in vitro* stops short of the infective stages and thus material for study can only be obtained from the salivary glands of infected tsetse flies. This must limit such investigations at present. However, it may be possible to study the transition in the reverse direction as bloodstream trypanosomes revert to insect forms when removed from the blood and incubated in culture medium. The previous results described demonstrate cytochrome only in the *lewisi* group and in several culture forms. Therefore trypanosomes of the *vivax, congolense* and *brucei* groups, in view of their higher respiratory rate, must contain a different and more efficient respiratory system. There is considerable evidence that the African trypanosomes utilize glucose by means of an E-M-P system (Marshall, 1948; Grant and Fulton, 1957) with formation of pyruvate. It is known that in this pathway DPN is reduced during the degradation glucose to pyruvate. As very little lactic acid is produced by these trypanosomes and they are normally highly aerobic, the DPNH formed must be reoxidized to DPN by a pathway involving molecular oxygen. A search was therefore made for a DPNH oxidase system as a first step in the elucidation of the

nature of the terminal respiratory pathway operative in these trypanosomes. In these preliminary studies suspensions of *T. rhodesiense* were used because of their ready availability in a pure state.

Incubation of homogenates of *T. rhodesiense* with substrate amounts of DPNH led to a marked increase in utilization of oxygen over that use by the homogenate alone. Oxidation of DPNH at room temperature was followed spectrophotometrically at 340 mμ by adding 0.1 ml of broken trypanosome suspension to 3 ml of phosphate buffered saline (pH 7.4) containing 0.1 micromole DPNH. In a quantitative experiment 6.1 micromoles of DPNH were oxidized and 2.8 micromoles of $O_2$ were taken up approximating to the stoichiometric ratio $2DPNH_2 + O_2 = 2DPN + 2H_2O$. In the reaction carried out at room temperature in the spectrophotometer (see Fig. 1) the addition of alcohol dehydrogenase and alcohol at the end of the reaction restored the peak at 340 mμ to 94 % of its original value, showing that DPNH was, indeed, being oxidized to DPN and not destroyed by nucleosidases or pyrophosphatases in the trypanosomes. Boiling the homogenate for 5 minutes completely abolished the reaction. It was also slightly inhibited by addition to the cuvette of iodoacetate ($3.3 \times 10^{-3}$M) or of undecane diamidine ($10^{-3}$M), but not by $10^{-2}$M cyanide, arsenite or fluoride.

Peroxide was not detected as a product of the reaction and tests for peroxidase activity in the homogenate by the addition to it of *p*-phenylene diamine or o-tolidine in the presence of peroxide were negative. It is seen therefore that the DPNH oxidase system does not involve the usual cytochrome pathway neither, apparently, does it contain flavo-protein oxidases because oxidases of this type are usually characterised by the formation of peroxide. *T. rhodesiense* is extremely sensitive to peroxide and thus accumulation of it cannot normally occur. Catalase is absent, but it is possible that a coupled DPNH oxidase-DPNH peroxidase system may be operative. Such a system in *Streptococcus faecalis* has been described by Dolin (1953), but so far we have been unable to find any evidence of such a coupled system in *T. rhodesiense*. The addition of peroxide does not accelerate the oxidation of DPNH by homogenates of the parasite, neither does excess catalase slow it. We have been unable to detect any peroxidative activity of the homogenate towards dyes. The oxidase system is not markedly affected by sulphydryl inhibitors, so its pres-

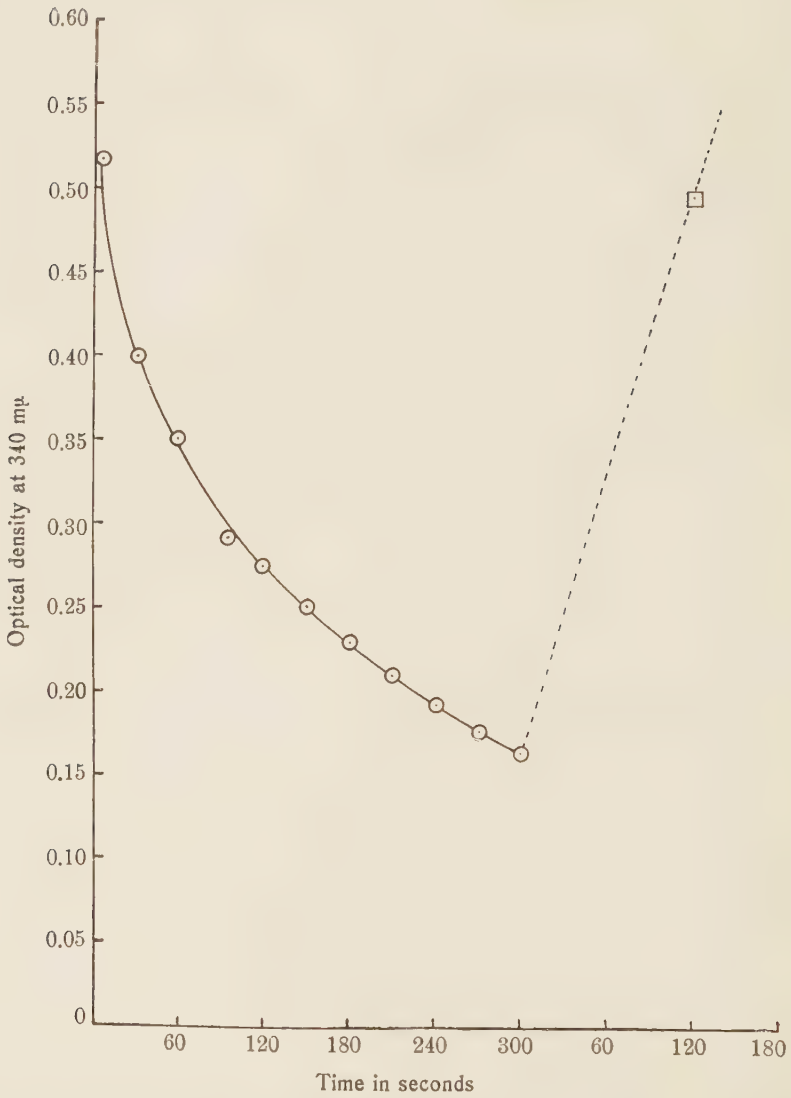


Fig. 1 — Oxidation of DPNH with fall in absorption by homogenate of *T. rodhesiense* and reconversion of DPN formed to DPNH by addition of alcohol dehydrogenase and substrate was followed in the spectrophotometer. The cuvette contained 0.26 micromoles DPNH in 3.0 ml phosphate buffered saline. The blank continaed saline only. 1.8 mg dried homogenate in 0.2 ml buffered saline was added to both cuvettes. After 5 minutes 2.0 ml mixture was removed from each cuvette and diluted with 1 ml M/10 pyrophosphate buffer at pH 9, 0.1 ml 95 % alcohol and 0.05 ml suspension of alcohol dehydrogenase was added. The absorption was read after 2 minutes and corrections made for dilution in calculating amount of DPNH now present

ence does not explain the marked sensitivity of the African trypanosomes to such inhibitors. Preliminary attempts to purify the oxidase system have been unsuccessful, but a knowledge of this pathway may be of chemotherapeutic importance as it seems to differ markedly from the pathways of DPNH oxidation so far reported in the mammalian host. However, a drug inhibiting this metabolic step in the trypanosomes may be of limited value, as it has been shown (Fulton and Spooner, 1956) that *T. rhodesiense* can survive, at least for some hours, anaerobically.

It has been pointed out by von Brand (1952) that compounds which react with -SH groups, such as arsenical drugs and iodoacetate, affect the respiration of different species of trypanosomes to a varying degree, in fairly good agreement with Hoare's classification. The respiration of *T. rhodesiense* is markedly inhibited by hydrogen peroxide and by traces of peroxide formed during auto-oxidation of thiols in air (Fulton and Spooner, 1956). It was thought that the inhibitory action of peroxide might be due to oxidation of -SH groups respiratory enzymes of trypanosomes. The effect of added or freshly generated («nascent») peroxide from cysteine autoxidation on the respiration of different species of trypanosomes was therefore measured to see if its effect is correlated with that of known thiol inhibitors. A concentration of $10^{-3}$M cysteine markedly inhibited the respiration of all the trypanosomes tested. There was, however, a considerable range of activity with some species, especially *S. oncopelti*. A concentration of $2.85 \times 10^{-4}$M peroxide inhibited all the organisms except *S. oncopelti*. However, when $50 \times 10^{-4}$M peroxide was used, the respiration of this organism was inhibited to the extent of 90 %. The use of cysteine and peroxide as selective agents therefore proved disappointing. It seems that the whole genus is very sensitive to peroxide, suggesting that none of the trypanosomes contain catalase. Attempts to identify this enzyme in the parasites in the past have always met with negative results (Krijgsman, 1936; Harvey, 1949; Ryley, 1955; Fulton and Spooner, 1956). It does not seem, as was thought possible, that peroxide is toxic to the organisms because it oxidizes thiol groups of trypanosome enzymes. It affects all species to a similar degree whereas thiol inhibitors markedly inhibit only the African trypanosomes (von Brand, 1951).

As we have emphasised, the bloodstream forms of the *vivax, congolense* and *brucei* groups of trypanosomes are characterised by the loss of the cytochrome type of terminal respiratory pathway found in monogenetic trypanosomes, the bloodstream forms of the *lewisi* group and the insect stages of all groups. However, as Moulder (1950) has pointed out, a new respiratory enzyme system has replaced the cytochrome system, and judging from the high $QO_2$ of the African trypanosomes, this is capable of functioning at a faster rate than the heavy-metal system. It is difficult to interpret this in the light of current thought about the evolution of the mammalian trypanosomes. It is generally considered that these parasites have descended from the monogenetic insect flagellates and Hoare (1957) maintains that while the *lewisi* group trypanosomes are the most primitive, those of the *brucei* group are the most recent, and should, therefore, be less well adapted parasites. However, the arisal of a new and efficient oxidase system would seem to indicate a marked adaptation to a parasitic existence conferring great advantage on a rapidly dividing parasite with high energy demands.

Full details of this work are to be published elsewhere.

## BIBLIOGRAPHY


Baernstein, H. D. — *Ann. N. Y. Acad. Science,* **56**: 983-994, 1951.

Baernstein, H. D., & Tobie, E. J. — *Fed. Proc.,* **10**: 159, 1951.

Von Brand, T. — *Biochemistry & Physiology of Protozoa.* Vol. 1. Ed. A. Lwoff, New York, Acad. Press, Inc., 1951.

Von Brand, T. — *Chemical Physiology of Endoparasitic Animals,* New York, Acad. Press. Inc., 1952.

Von Brand, T., & Tobie, E. J. — *J. Cell Compar. Physiol.,* **31**: 49-68, 1948.

Von Brand, T., Tobie, E. J., & Mehlman, B. — *J. Cell Compar. Physiol.,* **35**: 273-300, 1950.

Desowitz, R. S. — *Exper. Parasitol.,* **5**: 250-259, 1956.

Dolin, M. I. — *Arch. Biochem., Biophys.,* **46**: 483-485, 1953.

Fulton, J. D., & Spooner, D. F. — *Biochem. J.,* **63**: 475-481, 1956.

Fulton, J. D., & Spooner, D. F. — *Ann. trop. Med. Parasitol.,* **51**: 417-421, 1957.

Grant, P. T., & Fulton, J. D. — *Biochem. J.,* **66**: 242-250, 1957.

Harvey, S. C. — *J. Biol. Chem.,* **179**: 435-453, 1949.

Hoare, C. A. — *Veterinary Reviews and Annotations,* **3**: 1-13, 1957.

Keilin, D., & Hartree, E. F. — *Nature,* **164**: 254-259, 1949.

Krijgsman, B. J. — *Zeit. vergleich. Physiol.,* **23**: 663-711, 1936.

MARSHALL, P. B. — *Brit. J. Pharmacol.*, **3**: 1-7, 1948.
MOULDER, J. W. — *J. Parasitol.*, **36**: 193-200, 1950.
RYLEY, J. F. — *Biochem. J.*, **49**: 577-585, 1951.
RYLEY, J. F. — *Ibid.*, **59**: 353-361, 1955.
RYLEY, J. F. — *Ibid.*, **62**: 215-222, 1956.
SEAMAN, G. R. — *Exper. Parasitol.*, **5**: 138-148, 1956.
SMITH, L. — *Bact. Reviews*, **18**: 106-130, 1954.

# NUEVA TRYPANOSOMIASIS HUMANA DE LA REGIÓN NEOTRÓPICA PRODUCIDA POR EL *TRYPANOSOMA RANGELI* TEJERA, 1920, CON ESPECIAL REFERENCIA A VENEZUELA

ENRIQUE TEJERA

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Hacen 38 años, uno de nosotros (Tejera, 1920) describió con el nombre de *Trypanosoma* (o *Crithidia*) *rangeli* un flagelado con predominio de formas crithidias en el intestino del *Rhodnius prolixus* de Venezuela, principal transmisor de la enfermedad de Chagas en el país, sugiriendo para la fecha del hallazgo que el parásito podría tener un huésped vertebrado. El nuevo trypanosoma fué comprobado por primera vez en sangre humana por Romeo de León en Guatemala, en 1936, quien lo consideró como especie nueva denominándolo *Trypanosoma guatemalense*. Uno de nosotros (Pifano, 1948) demostró la identidad de este trypanosoma con el *Trypanosoma rangeli*. El trypanosoma comprobado por Groot, Renjifo y Uribe en habitantes del Valle del río Ariari, Colombia, en 1950, y denominado por estos autores *Trypanosoma ariarii*, corresponde igualmente al *Trypanosoma rangeli* de Tejera.

Hasta el presente, la trypanosomiasis *rangeli* en el hombre ha sido comprobada con caracter endémico en Venezuela, Colombia y Guatemala, existiendo una superposición entre su distribución geográfica y la de la enfermedad de Chagas, con la cual presenta aspectos epidemiológicos comunes. Existen, no obstante, focos de trypanosomiasis *rangeli* puros, tanto en Colombia como en Venezuela.

En la sangre periférica del hombre y en la de animales infectados experimentalmente (con heces o líquido celómico de triatomas, cultivos) o con infección natural, el *Trypanosoma rangeli* se caracteriza por su gran tamaño (26 $\mu$ a 34 $\mu$), blefaroplasto en forma de gránulo situado a distancia de la extremidad posterior del cuerpo del parásito, núcleo de situación media o ligeramente desplazado hacia la unión

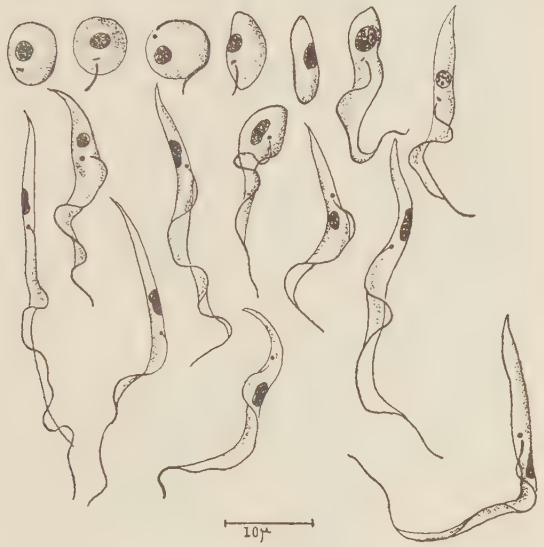


Fig. 1 — *Trypanosoma rangeli* Tejera, 1920. Contenido intestinal del *Rhodnius prolixus* de Venezuela. Según el Dr. Enrique Tejera, dibujo original. «Un nouveau flagelle de *Rhodnius prolixus, Trypanosoma* (o *Crithidia*) *rangeli* n. sp.», *Bulletin de la Société de Pathologie Exotique,* XIII (7); 527-530, 1920

del tercio medio con el tercio anterior del cuerpo, membrana ondulante bien desarrollada y flagelo libre muy largo. No han podido ser observadas formas de division evolutivas de este trypanosoma en la sangre del vertebrado, así como tampoco formas leishmanias en los tejidos. En el tubo digestivo del transmisor (*Rhodnius prolixus* en Venezuela, Colombia y Guatemala; *Triatoma dimidiata* en Guatemala), el *Trypanosoma rangeli* presenta los siguientes estadios evolutivos: (1) formas piriformes, que se encuentran preferentemente en el intestino anterior, de aproximadamente 7 $\mu$ de longitud, sin flagelo o con

un flagelo muy corto, aparentemente recién formado; (2) crithidias cortas, semejantes a leptomonas, con membrana ondulante todavía no bien desarrollada; (3) crithidias largas, especialmente en el intestino posterior, de gran tamaño (31-70 $\mu$ y a veces más), extremidad pos-

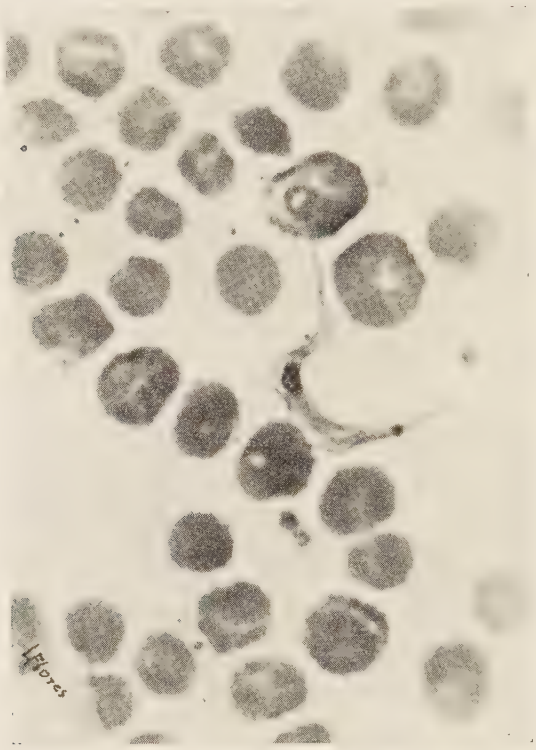

Fig. 2 — *Trypanosoma rangeli* Tejera, 1920. Sangre periférica del hombre

terior del cuerpo del parásito larga y fina, blefaroplasto puntiforme muy cerca del núcleo y membrana ondulante bien desarrollada; (4) formas trypanosomas, especialmente en el intestino posterior y ampolla rectal, habitualmente escasas, de 33 $\mu$ a 53 $\mu$ de longitud, con cuerpo delgado y extremidades finas, blefaroplasto en forma de gránulo situado a cierta distancia de la extremidad posterior del cuerpo del parásito, membrana ondulante bien desarrollada y flagelo libre muy largo. El *Trypanosoma rangeli* invade la cavidad celómica del tria-

tomidio a partir del 15.° día de la infección y realiza un ciclo anterior en las glándulas salivares del insecto, con la presencia de formas

Fig. 3 — *Trypanosoma rangeli* Tejera, 1920. Sangre periférica de ratón blanco joven a los 10 días de inoculado con cultivo de caso humano. Dibujo en cámara clara

trypanosomas en la saliva. La transmisión se realiza por picadura (Groot, 1952; Pifano, 1949). El *Trypanosoma rangeli* es patógeno para el *Rhodnius prolixus,* siendo causa de muerte en ninfas y larvas especialmente, a medida que aumenta la parasitemia en el líquido

celómico. Los ejemplares infectados que sobreviven hasta adultos únicamente presentan infección intestinal. La inoculación directa del *Trypanosoma rangeli* al hemocele demuestra que la mortalidad no es debida a la perforación de la pared intestinal por el trypanosoma.

Fig. 4 — *Trypanosoma rangeli* Tejera, 1920. Sangre periférica de cachorro de perro a los 7 días de inoculado con cultivo de caso humano. Dibujo en cámara clara

La patogenicidad está siempre asociada a la invasión del celoma por los parásitos en donde se encuentran todos los estadios evolutivos con formas leishmanias libres o incluidas en hemocitos. En condiciones experimentales, el *Trypanosoma rangeli* evoluciona en *Triatoma dimidiata capitata* y *Cavernicola pilosa* (Groot, 1952), así como también en *Cimex lectularius* (Grewal, 1957). Coutinho y Nussenswing

(1952) han logrado la infección experimental de *Rhodnius prolixus, Triatoma infestans, Triatoma vitticeps* y *Panstrongylus megistus,* por inoculación de los trypanosomas en el abdomen de los triatomas. Nosotros hemos logrado la infección experimental por hematofagismo

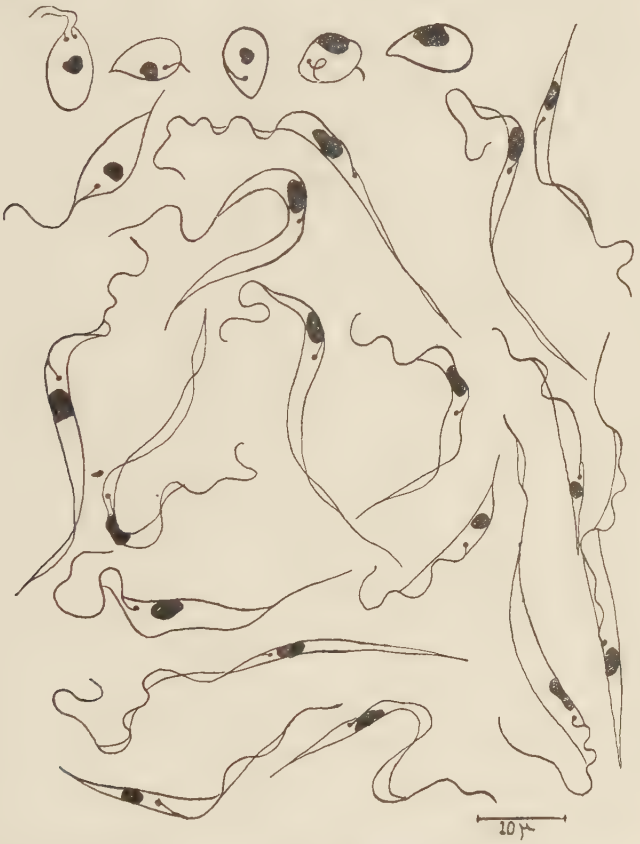


Fig. 5 — *Trypanosoma rangeli* Tejera, 1920. Contenido intestinal del *Rhodnius prolixus* de Venezuela. Infección natural. Dibujo en cámara clara

de *Triatoma infestans, Triatoma maculata, Triatoma brasiliensis, Triatoma palidipennis* y *Rhodnius pictipes.*

El *Trypanosoma rangeli* se cultiva fácilmente en medios con gelosa sangre (NNN, gelosa nutritiva glucosada según Davis, Senekjie, Nöller y otros), así como también en el medio de Razgha-Rei-

chenow. Algunos animales de laboratorio (ratón blanco recién nacido, cachorro de perro, monos) son sensibles a las inoculaciones con *Trypanosoma rangeli*. Habitualmente la parasitemia en los animales ino-

Fig. 6 — *Trypanosoma rangeli* Tejera, 1920. Formas evolutivas en el contenido intestinal de *Rhodnius prolixus* de cria alimentado en caso humano de trypanosomiasis *rangeli*. Dibujo en cámara clara

culados es escasa y los trypanosomas circulan en la sangre periférica en forma discontinua e irregular durante dos a cuatro semanas para luego desaparecer. La infección latente es posible comprobarla en estos animales mediante el xenodiagnóstico y el hemocultivo. Un *Cebus apella* inoculado experimentalmente por nosotros con *Trypa-*

*nosoma rangeli* de proveniencia humana (xenodiagnóstico) mostró escasos trypanosomas en sangre periférica en las dos semanas que siguieron a la inoculación, habiéndose comprobado la existencia de una infección latente durante cuatro años mediante xenodiagnósticos y hemocultivos practicados periódicamente. En personas que han abandonado el área endémica hemos comprobado la existencia de infec-

Fig. 7 — *Trypanosoma rangeli* Tejera, 1920. Formas evolutivas en el jugo de la trompa del *Rhodnius prolixus* de Venezuela. Infección natural. Dibujo en cámara clara

ciones latentes por xenodiagnósticos y hemocultivos dos a cuatro años después, con la seguridad de no haberse re-infectado.

Hasta el presente, hemos comprobado en Venezuela 670 casos de trypanosomiasis *rangeli*, especialmente en niños de 5 a 14 años, de los cuales 45 por el examen directo de la sangre (gota gruesa), 146 por hemocultivo y 479 por el xenodiagnóstico. En 120 casos en que hemos practicado simultáneamente el xenodiagnóstico y el hemocultivo, hemos encontrado hemocultivo positivo y xenodiagnóstico negativo en 20 casos (16,66 %) y xenodiagnóstico positivo y

hemocultivo negativo en 12 casos (10 %). En el material humano hasta ahora examinado no hemos encontrado síntomas que indiquen una acción patógena del protozoario. Existen en Venezuela focos de trypanosomiasis *rangeli* puros y áreas endémicas de enfermedad

Fig. 8 — *Trypanosoma rangeli* Tejera, 1920. Cultivo de 6 días en gelosa nutritiva glucosada según Davis. Hemocultivo de caso humano. Dibujo en cámara clara

de Chagas asociada a trypanosomiasis *rangeli*. En um mismo indivíduo hemos comprobado la presencia de *Schizotrypanum cruzi* y *Trypanosoma rangeli* en forma simultánea o sucesiva, mediante xenodiagnósticos y hemocultivos practicados periódicamente. Esto nos lleva a establecer que en una zona donde las dos trypanosomiasis revistan

carácter endémico no es posible descartar una de ellas por el hecho de haberse comprobado la otra.

Fig. 9 — *Trypanosoma rangeli* Tejera, 1920. Cultivo de 9 días en gelosa nutritiva glucosada según Davis. Hemocultivo de caso humano. Dibujo en cámara clara

La infección natural del *Rhodnius prolixus* por el *Trypanosoma rangeli* en las zonas rurales del país constituye un xenodiagnóstico natural que indica la existencia de la trypanosomiasis en un vertebrado, hombre o animales domésticos próximos al hombre, que ha constituído la fuente de alimentación de los triatomas. Hemos comprobado con mucha frecuencia la infección natural del perro por el

*Trypanosoma rangeli* y en menor proporción en el gato. Existe una evidente superposición de la infección humana con la infección natu-

Fig. 10 — *Trypanosoma rangeli* Tejera, 1920. De *Didelphis marsupialis* de Venezuela. *A*, formas trypanosomas en sangre periférica; *B*, formas de desarrollo en *Rhodnius prolixus* (xenodiagnóstico); *C*, formas de desarrollo en medios de cultivo (gelosa nutritiva según Davis). Dibujo en cámara clara

ral del *Rhodnius prolixus* y con la de los animales domésticos naturalmente infectados. Los siguientes resultados de una investigación de campo y de laboratorio realizada en un sector del Estado Yaracuy, Venezuela, ilustra la superposición de los factores epidemiológicos

referidos. El estudio de 3.397 ejemplares de *Rhodnius prolixus,* mediante el examen del contenido intestinal en fresco y coloreado según Giemsa, sobre un total de 8.564 especímenes capturados en 216 viviendas rurales de la zona investigada, ha demostrado las siguientes modalidades de infección trypanosomiásica natural: infectados con trypanosomas: 1.892 (55,07 %); infectados con *Trypanosoma rangeli:* 775 (40,96 %); infectados con *Schizotrypanum cruzi:* 800 (42,28 %); infección mista *Trypanosoma rangeli-Schizotrypanum cruzi:* 317 (16,75 %). En la misma zona se examinaron un total de 1.124 personas mediante el xenodiagnóstico, preferentemente niños de 5 a 10 años, encontrándose los resultados siguientes: infectados con trypanosomas: 383 (34,07 %); infectados con *Trypanosoma rangeli:* 200 (52,22 %); infectados con *Schizotrypanum cruzi:* 145 (37,86 %); infección mixta *Trypanosoma rangeli-Schizotypanum cruzi:* 38 (9,92%). De 63 perros examinados simultáneamente con el material humano mediante xenodiagnóstico, 28 se encontraron infectados con trypanosomas (44,50 %). Entre los infectados, 9 estaban parasitados con *Trypanosoma rangeli* (32,14 %); 15 con *Schizotrypanum cruzi* (14,29 %), y 4 con infección mixta *Trypanosoma rangeli-Schizotrypanum cruzi* (14,29 %).

Además de la trypanosomiasis *rangeli* de carácter rural, hemos verificado algunos hallazgos que indican la proveniencia silvestre de esta parasitosis. Hemos comprobado la infección natural por el *Trypanosoma rangeli* del *Panstrongylus geniculatus* capturados en cuevas y en un espécimen de *Didelphis marsupialis.* Groot y colaboradores, en 1951, relatan también el hallazgo en condiciones naturales del *Trypanosoma rangeli* en el *Cebus fatuelles.* Es de importancia señalar que el *Trypanosoma myrmecophagae* Floch, Lajudie y A. Abonnenc, 1941, del *Myrmecophaga trydactyla,* el *Trypanosoma diasi* Deanne y Martins, 1951, el trypanosoma del *Cebus apella apella,* los trypanosomas de *Cebus* sp., de *Atelles paniscus* y de *Didelphis marsupialis* descritos por Floch y colaboradores en 1949 y el *Trypanosoma barnolae* Torrealba y colaboradores, 1955, pueden evolucionar en el intestino del *Rhodnius prolixus,* en el cual presentan aspectos parecidos al *Trypanosoma rangeli.* La relación entre estos trypanosomas no está aclarada y el hecho de que evolucionan en triatomidios permite agruparlos en un complejo biológico que Groot y nosotros denominamos «grupo *rangeli*».

El método más práctico para el diagnóstico de la trypanosomiasis *rangeli* es por el hemocultivo y el xenodiagnóstico, practicados en forma simultánea, ya que disponemos de casos con hemocultivo positivo que escaparon al xenodiagnóstico (16,66 %) y casos con xenodiagnóstico positivo que escaparon al hemocultivo (10 %). La reacción de desviación del complemento con antígenos elaborados con cultivos de *Trypanosoma rangeli* es positiva en casos de trypanosomiasis *rangeli* y negativa en casos de enfermedad de Chagas (Pifano, Scorza y Sequera, 1956, ined.). Las reacciones serológicas practicadas en casos de trypanosomiasis *rangeli* con antígenos de cultivos de *Schizotrypanum cruzi* han sido siempre negativas. Los resultados de las pruebas cruzadas descartan la posibilidad de reacciones de grupo en estas trypanosomiasis.

La trypanosomiasis *rangeli* constituye en Venezuela y en los otros países donde se ha comprobado su existencia una *trypanosomiasis de interferencia,* que debe ser tomada en cuenta para la correcta interpretación de las encuestas epidemiológicas de reconocimiento sobre la enfermedad de Chagas en una región fundamentadas en el estudio de la infección natural de triatomidios por trypanosomas y en la prueba del xenodiagnóstico. Recomendamos que se practique, además del examen en fresco, la coloración del contenido intestinal de triatomidios, complementada con inoculaciones a animales de laboratorio, cuando en el transcurso de investigaciones sobre enfermedad de Chagas se encuentran reduvídeos infectados con trypanosomas que presenten características insuficientes para identificarlos con *Schizotrypanum cruzi.*

## RESUMEN

Una nueva trypanosomiasis humana de la región neotrópica producida por el *Trypanosoma rangeli* Tejera, 1920, reviste carácter endémico en zonas rurales de Venezuela, Colombia y Guatemala, siendo probable su distribución más extensa en otros países del trópico americano. El parásito fué descrito hacen 38 años por uno de los autores (Tejera) con el nombre de *Trypanosoma* (o *Crithidia*) *rangeli* en el contenido intestinal del *Rhodnius prolixus* de Venezuela, principal transmisor de la enfermedad de Chagas en el país, sugiriendo para la fecha de su hallazgo que el protozoario podría tener un huésped vertebrado. Las primeras formas en sangre periférica del hombre fueron observadas por Romeo de León en Guatemala en 1936.

Los trypanosomas descritos como *Trypanosoma guatemalense* Romeo de León, 1946, y *Trypanosoma ariarii* Groot, Renjifo y Uribe, 1950, corresponden al *Trypanosoma rangeli* Tejera, 1920. En la sangre periférica del hombre el *Trypanosoma rangeli* tiene 26 $\mu$ a 34 $\mu$ de longitud, blefaroplasto en forma de gránulo situado a distancia de la extremidad posterior del cuerpo del parásito, núcleo de situación media o ligeramente desplazado hacia la extremidad anterior del cuerpo, membrana ondulante bien desarrollada y flagelo libre muy largo. No han sido observadas formas de división de este trypanosoma en sangre periférica ni formas leishmanias en los tejidos. En el insecto transmisor *(Rhodnius prolixus)* realiza un ciclo intestinal (crithidias cortas y de gran tamaño, formas trypanosomas) con invasión de la cavidad celómica y presencia de trypanosomas en las glándulas salivares y en la saliva. La transmisión se realiza por picadura, distinto a lo que ocurre con el *Schizotrypanum cruzi*. En algunos animales de laboratorio, especialmente ratones recién nacidos y cachorros de perro, las inoculaciones determinan una parasitemia escasa, circulando los trypanosomas en sangre periférica en forma discontinua e irregular durante dos a cuatro semanas para luego desaparecer y pasar al estado de parasitismo latente demostrable mediante el hemocultivo y el xenodiagnóstico. Es fácilmente cultivado en medio con agar sangre (NNN, Davis, Nöler y otros) y en el medio de Rhazga-Reichenow. Hasta el presente hemos comprobado en Venezuela 670 casos de trypanosomiasis *rangeli*, especialmente en niños de 5 a 14 años, de los cuales 45 por el examen directo de la sangre (gotas gruesas), 146 por el hemocultivo y 479 por el xenodiagnóstico. En el material humano hasta ahora examinado no se han comprobado síntomas que indiquen una acción patógena del protozoario. Existe en Venezuela una superposición entre la trypanosomiasis *rangeli* humana y la infección natural del *Rhodnius prolixus* y del perro por el *Trypanosoma rangeli*. La fuente de infección de los triatomidios es el hombre o los animales domésticos próximos al hombre parasitados por el trypanosoma. Hemos comprobado en el país focos puros de trypanosomiasis *rangeli* y áreas de *enfermedad de Chagas* asociada a dicha trypanosomiasis. El diagnóstico de la trypanosomiasis *rangeli* se realiza por el xenodiagnóstico y el hemocultivo, siendo recomendable emplear simultâneamente ambos procedimientos. No existe en la trypanosomiasis *rangeli* una reacción serológica de grupo con antígenos elaborados con cultivos de *Schizotrypanum cruzi* (Pifano, Scorza y Sequera, ided.). Algunos animales silvestres *(Cebus fatulles* en Colombia y *Didelphis marsupialis* en Venezuela) han sido encontrados infectados en condiciones naturales con el *Trypanosoma rangeli*. La infección natural ha sido también comprobada en un triatomidio silvestre (*Panstrongylus geniculatus* de Venezuela). La relación entre otros trypanosomas de animales silvestres que evolucionan en triatomidios y el *Trypanosoma rangeli* no está aclarada e integran al parecer un complejo biológico que denominamos «grupo *rangeli*». La trypanosomiasis *rangeli* constituye en Venezuela y en los otros países donde se ha comprobado su existencia una *trypanosomiasis de interferencia*, que debe ser tomada en cuenta para la correcta interpretación de las encuestas epidemiológicas de reconocimiento sobre enfermedad de Chagas en una región, fundamentadas en el estudio de la infección natural de triatomidios por trypanosomas y en la prueba del xenodiagnóstico. Recomendamos que se practique, además del examen en fresco,

la coloración del contenido intestinal de los triatomidios cuando en el transcurso de investigaciones sobre enfermedad de Chagas se encuentren reduvídeos infectados con trypanosomas que presenten características insuficientes para identificarlos con el *Schizotrypanum cruzi*.

## SUMMARY

A new human trypanosomiasis in the neotropical region produced by *Trypanosoma rangeli* Tejera, 1920, is endemic in rural areas of Venezuela, Colombia and Guatemala and its distribution is probably wider in other tropical countries of the Americas.

The parasite was described 38 years ago by one of us (Tejera) with the name of *Trypanosoma* (or *Crithidia*) *rangeli*. It was found in the intestinal contents of the *Rhodnius prolixus*, the main vector of Chagas' disease in Venezuela suggesting as the date of discovery that a vertebrate host should exist for the protozoan.

The first forms were observed in human periphereal blood by Romeo de León in 1936, in Guatemala.

The species described as *T. guatemalense* Romeo de León, 1946, and *T. ariari* Groot, Rengifo, and Uribe, 1950, are synonimous with *T. rangeli* Tejera, 1920.

In human periphereal blood the lenght of *T. rangeli* is 26 $\mu$ to 34 $\mu$. The blepharoplast has the form of a granule situated far from the posterior extreme of the parasite and the nucleus at the midle, or displaced to the anterior part. It has a well developed undulant membrane and flagellum free and long.

No division forms have been observed in periphereal blood, nor leishmania forms in the tissues.

The intestinal cycle develops in the vector *(R. prolixus)* long and short crithidial and trypanosomes forms with invasion of the body cavity and presence of trypanosomes forms in the salivary glands and saliva.

The transmission is by biting, different from the one of *S. cruzi*.

In new born mice and puppies inoculated at the laboratory the number of parasites circulating in the blood is scarce, their appearance is irregular and after two or four weeks the animals have a latent parasitism only demonstrated by blood culture and xenodiagnosis.

The parasite is easily cultivated in blood media (NNN, Davis, Noller and others) and also in the Rhazga Reichenow's media.

Until today, 670 cases of *T. rangeli* have been discovered in Venezuela, 45 by thick film, 146 by blood culture and 479 by xenodiagnosis; the findings are predominant in the age group 5 to 14.

In all cases examined no pathology atributed to *T. rangeli* has been found.

In Venezuela a closed relation exists between the infection of humans, *Rhodnius prolixus* and dogs by *T. rangeli*. Man and domestic animals are the source of infection.

Natural infection of selvatic animals like *Cebus fatuelles* in Colombia and *Didelphis marsupialis* in Venezuela has been demonstrated. Also in a selvatic triatoma *Panstrongylus geniculatus* in Venezuela.

We demonstrated areas in Venezuela where only *T. rangeli* exists and also other areas where Chagas and *T. rangeli* are together.

The relation of *T. rangeli* with other trypanosoma of selvatic animals is not well established and the existence of biological complex that we call the «*rangeli* group» is possible.

We recommend for diagnosis of *T. rangeli* simultaneous use of blood culture and xenodiagnostic procedure. There is no serological reaction for *T. rangeli* with antigen prepared with cultures of *S. cruzi* (Pifano, Scorza, and Sequera, not yet published).

The *rangeli* trypanosomiasis represented in Venezuela and in other countries is an *interference disease* that must be considered for the interpretation of epidemiological surveys based on the study of *Triatomidae* and xenodiagnostic tests. Besides the direct examination of the intestinal content of *Triatomidae*, we recommend that staining procedures should be used for differential diagnosis of *trypanosoma*, without sufficient characteristics to classify them as *S. cruzi*.

## BIBLIOGRAFÍA


1. TEJERA, E. — «Un nouveau flagelle de *Rhodnius prolixus, Trypanosoma* (ou *Crithidia*) *rangeli* n. sp.», *Bulletin de la Société de Pathologie Exotique*, **13**: (7): 527-530, Paris, 1920.
2. FLOCH, H., DE LEJUDIE, P., et ABONNENC, E. — *Un trypanosome d'un édenté* (Mimecofagae tridactyla) *suceptible d'évoluer dans le tube digestive de* R. prolixus. Public. n.° 25 de l'Institut Pasteur de la Guyane Française. Guayana Francesa, S.A., 1941.
3. DE LEÓN, ROMEO — *Contribución al estudio de la enfermedad de Chagas en Guatemala*. Trabajo presentado al Primer Congreso Interamericano de Medicina de Río de Janeiro, Brasil, 1 vol., Guatemala, 1946.
4. PIFANO C., FÉLIX — «Nueva trypanosomiasis humana de la región neotrópica producida por el *Trypanosoma rangeli*». Trabajo presentado al IV Congreso Internacional de Medicina Tropical y Malaria, reunido en Washington D.C., mayo 10-18, 1948. *Proceedings of the Fourth International Congress on Tropical Medicine and Malaria*, p. 1166, Washington, U.S.A., 1948.
5. PIFANO C., FÉLIX, MAYER, M., MEDINA, R., y BENAIM PINTO, H. — «Primera comprobación del trypanosoma *rangeli* en el organismo humano por cultivo de sangre periférica», *Archivos Venezolanos de Patología Tropical y Parasitología Médica*, **1** (1): 1-31, Caracas, Venezuela, 1948.
6. PIFANO C., FÉLIX, y colaboradores — «Nouvelle trypanosomiase humaine de la region neotropicale produite par le *Trypanosoma rangeli* Tejera, 1920», *Bulletin de la Société de Pathologie Exothique*, **41** (11-12): 671-680, Paris, 1948.
7. FLOCH, H., y ABONNENC, E. — *Trypanosome de* Didelphis marsupialis *dont les formes metacicliques chez* R. prolixus *et* rubrofaciata *ressemblent à celles*

*de* T. mimecophagae. Public. n.° 171 de l'Institut Pasteur de la Guyane et du territoire de l'Inini, Guayana Francesa, S.A., 1948.

8. Floch, H., et Abonnenc, E. — *Sur un trypanosomide de singe infectant naturellement la cavité générale de* Triatoma rubrofasciata *et celle de* Rhodnius prolixus. Public. n° 201 de l'Institut Pasteur de la Guyane et du territoire de l'Inini, Guayana Francesa, S.A., 1949.
9. Pifano C., Félix, y col. — «Estado actual de las investigaciones en Venezuela sobre una nueva trypanosomiasis humana de la región neotrópica producida por el *Trypanosoma rangeli*». Trabajo presentado en la Primera Reunión Panamericana de Enfermedad de Chagas en Tucumán. Argentina (julio, 11-16, 1949). *Archivos Venezolanos de Patología Tropical y Parasitología Médica,* **1** (2): 135-152, Caracas, Venezuela, 1949.
10. Pifano C., Félix, y Mayer, Martín — «Hallazgo de formas evolutivas del *Trypanosoma rangeli* en el jugo de la trompa de *Rhodnius prolixus* de Venezuela», *Archivos Venezolanos de Patología Tropical y Parasitología Médica,* **1** (2): 153-158, Caracas, Venezuela, 1949.
11. Floch, H., et Abonnenc, E. — *Sur un trypanosome et un schizotrypanosome de singe évoluant chez les triatomides et inoculables aux animaux de laboratoires, leurs rapport avec* T. rangeli *Tejera,* 1920. Public. n.° 187, Institut Pasteur de la Guyane y du territoire de l'Inini, Guayana Francesa, S.A., 1949.
12. Groot, H., Renjifo, S., y Uribe, C. — «*Trypanosoma ariari* n. sp. *from man* found in Colombia», *The American Journal of Tropical Medicine,* **31** (6), U.S.A., 1951.
13. Coutinho, J. O., e Nussenzweig — «Infecção experimental de triatomídios pelo *T. rangeli* Tejera, 1920», *Folia Clínica e Biológica,* **18** (3): 181-188, 1952.
14. Groot, H. — «Fither observations on *Trypanosoma ariari* of Colombia, S.A.», *The American Journal of Tropical Medicine and Hygiene,* **1** (4), U.S.A., 1952.
15. Deane, L. M., y Martins, R. — «Sobre um tripanossoma encontrado em macaco da Amazónia e que evolui em triatomíneos», *Revista Brasileira de Malariologia e Doenças Tropicais,* vol. IV, Rio de Janeiro, 1952.
16. Pifano C., Félix — «Nueva trypanosomiasis humana de la región neotrópica producida por el *Trypanosoma rangeli,* con especial referencia a Venezuela», *Archivos Venezolanos de Patología Tropical y Parasitología Médica,* **2** (2): 89-120, Caracas, Venezuela, 1954.
17. Jaimes Berti, Jaime — *Estudios sobre trypanosomiasis americana por* Trypanosoma rangeli. Tesis de grado, 1 vol., pp. 1-37, Bogotá, Colombia, 1954.
18. Torrealba, J. F., Picardi, B., Ramos, I., Díaz Vasquez, A., y Torrealba, P. A. — «Breve nota sobre un nuevo trypanosoma infectante para triatominos comprobado en un mono en Venezuela: el *Cebus nigrivittatus*», *Gaceta Médica,* **63** (1-2): 2-8, Caracas, Venezuela, 1955.
19. Grewal, M. S. — «Pathogenicity of the *Trypanosoma rangeli* Tejera, 1920, in the invertebrate host», *Experimental Parasitology,* **6** (2), 123-130, 1957.

# ENFERMEDAD DE CHAGAS. ESTADO ACTUAL EN EL PERU

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La enfermedad de Chagas, considerada como vergonzante, ya que prolifera donde existe miseria e ignorancia (47), es una enfermedad eminentemente rural y semirural que predomina en pequeños caseríos, haciendas y pueblos donde la casa habitación, primitiva o rústica, está sobrepoblada y sus habitantes viven en marcada promiscuidad con chirimachas y animales domésticos que sirven de reservorio al agente etiológico del mal.

El 2 de diciembre de 1952, en la actuación central realizada en el Paraninfo del Ministerio de Salud Pública en Lima, con motivo de la celebración del Día Panamericano de la Salud, nos ocupámos de los aspectos epidemiológicos de esta enfermedad en América y especialmente en el Perú. Entonces señalámos que ella constituia un problema de salud pública entre nosotros y que si algunos médicos escépticos la consideraban como enfermedad exótica se debía a que las investigaciones eran escasas en número y solo se habían realizado en forma esporádica. Aprovechámos también la oportunidad para estimular a los investigadores nacionales y a las autoridades sanitarias para que se intensificasen los estudios sobre esta dolencia.

Despues de pocos años vemos con gran satisfacción que los científicos peruanos han ampliado nuestros conocimientos sobre su extensión y factores de diseminación, y el Ministerio de Salud Pública ha realizado algunas campañas profilácticas contra el insecto vector; pero es de desear que continuen tales investigaciones, porque los resul-

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tados obtenidos hasta hoy solo son un pálido reflejo de la magnitud que el problema tiene entre nosotros.

Se ha demostrado que la enfermedad de Chagas es endémica en todos los países limítrofes con el Perú, habiéndose comprobado la presencia de triatóminos, con elevados porcentajes de infección por *T. cruzi*, en Ecuador, Colombia, Brasil, Bolivia y Chile.

Varias especies de triatóminos que tienen especial importancia epidemiológica en la república del Ecuador han sido capturados últimamente en los departamentos del norte del Perú y el trasmisor más importante en Chile ingresó a nuestro país, posiblemente, durante la guerra de 1879, habiéndose dispersado por los departamentos del sur hasta llegar a Lima.

## HISTORIA

Los primeros informes en relación con la enfermedad de Chagas en el Perú se deben a Latreille, quien en 1811 señaló la presencia de triatóminos dentro de nuestras fronteras, al describir el *P. geniculatus* a base de especímenes de procedencia peruana.

Un siglo despues, en 1917, E. Escomel (10) encontró triatóminos, naturalmente infectados por *Trypanosoma cruzi*, en un valle del departamento de Arequipa y al año siguiente comunicó que el insecto capturado pertencía a la especie *Triatoma infestans* (11).

El primer caso de enfermedad de Chagas entre nosotros fue diagnosticado por E. Escomel (12), en 1919, al estudiar un paciente de 40 años que procedía del rio Tahuamanu, en Madre de Dios, y las primeras investigaciones referentes a animales reservorios del *Trypanosoma cruzi* las realizaron Ayulo y Herrer (2), en 1944, quienes encontraron infección natural por *T. cruzi* en 5,64 % de 443 cobayos y en 2,77 % de 36 perros examinados en el departamento de Arequipa.

La bibliografía sobre trypanosomiasis americana en el Perú es sumamente escasa entre los años 1811 y 1950, y es solo a partir de 1951 que nuestros investigadores ponen especial énfasis en su estudio, como una contribución al mejor conocimiento de una endemia que se extiende a casi la totalidad de los países del continente americano, desde los Estados Unidos de Norteamérica hasta la Patagonia Argentina, con miras a conseguir su erradicación.

## CASUÍSTICA PERUANA

El primer caso de enfermedad de Chagas en el Perú fue comunicado por el Dr. Edmundo Escomel (12), en el año 1919, y no se tuvo informes de otros hasta 1944, en que Ayulo y Herrer (2) publicaron los resultados de sus investigaciones en los valles de Victor, Sihuas y Quishuarani, en el departamento de Arequipa, y el hallazgo de un nuevo caso. En 1951 Uriel García (15) refirió 5 casos en Moquegua y el mismo año Cornejo Donayre y Lumbreras C. (5) informaron el hallazgo de 10 nuevos pacientes encontrados en el valle de Majes, del departamento de Arequipa.

Entre los años 1951 y 1957 se ha incrementado rápidamente la casuística, debido a que se ha realizado mayor número de encuestas epidemiológicas.

En 1954 Lazo Taboada y García-Durand (27) estudiaron un nuevo caso procedente de Arequipa y el mismo año Herrer (17) comunicó el hallazgo de 22 enfermos en la cuenca del Marañon. Al año siguiente, Lumbreras, Juana Arrarte y Brígida Guevara (34) diagnosticaron enfermedad de Chagas en dos pacientes del departamento de San Martin.

Los médicos peruanos F. y N. Náquira, que trabajan con el Prof. Amador Neghme en la Cátedra de Parasitología de la Escuela de Medicina de la Universidad de Chile, efectuaron en 1955 (41) una encuesta epidemiológica en la provincia de Tarata, del departamento de Tacna, la cual puso en evidencia la presencia de 45 nuevos enfermos.

En 1955 Herrer (20) encontró 3 casos en Moquegua y en 1956 el mismo autor (26) publicó 10 nuevos casos diagnosticados en Yantaló, departamento de San Martin.

Los hermanos F. y N. Náquira publicaron en 1957 (42) el resultado de sus nuevas investigaciones realizadas en Moquegua y Tacna durante el año 1956, demostrando que en 82 residentes de esas localidades la reacción de Guerreiro-Machado había sido positiva.

J. Montesinos, C. Náquira, J. Ordoñes, O. Paredes, V. Rondon, G. Silva, J. Vargas y A. Tejada, estudiantes de la Facultad de Medicina de Lima, han efectuado, bajo los auspicios de la Cátedra de Enfermedades Tropicales, una investigación sobre enfermedad de

Chagas en el valle de Sihuas, en el departamento de Arequipa. Este interesante trabajo ha revelado 22 nuevos casos con los que se ha incrementado la casuística nacional (39).

Se ha verificado la enfermedad de Chagas en los siguientes departamentos: Tacna, Moquegua, Arequipa, Madre de Dios, San Martin y Amazonas.

En el cuadro n.° 1 se puede apreciar, tanto la distribución de los 204 casos diagnosticados hasta la fecha en el Perú, como el método de diagnóstico empleado. Además, anotamos el autor y la fecha del hallazgo.

CUADRO N.° 1 — *Casos de enfermedad de Chagas diagnosticados en el Perú entre los años 1919 y 1957*

| Departamento | Año | Método de diagnóstico empleado | | | Total de casos | Autor |
|---|---|---|---|---|---|---|
| | | Directo sangre | Xenodiagnóstico | Fijación de comp. | | |
| Madre de Dios. . . . . | 1919 | 1 | | | 1 | E. Escomel (12). |
| Arequipa . . . . . | 1944 | 1 | | | 1 | Ayulo y Herrer (2). |
| | 1951 | | 10 | | 10 | Cornejo Donayre y Lumbreras Cruz (5). |
| | 1954 | 1 | | | 1 | Lazo Taboada y García-Durand (27). |
| | 1956 | | 22 | 5 | 22 | J. Montesinos, C. Náquira, J. Ordoñez, O. Paredes, V. Rojas, G. Sevillano, J. Vargas y A. Tejada (39). |
| | 1957 | | | | | |
| Moquegua . . . . . | 1951 | 1 | 5 | | 5 | Uriel García (15). |
| | 1951 | | 3 | | 3 | A. Herrer, (20). |
| Tacna. . . . . | 1955 | | 29 | 45 | 45 | F. y N. Náquira (41). |
| Tacna-Moquegua . . . . | 1957 | | 19 | 82 | 82 | F. y N. Náquira (42). |
| San Martin. . . . . | 1954 | | 2 | | 2 | Lumbreras, Arrarte y Guevara (34). |
| | 1956 | | 10 | | 10 | A. Herrer (26). |
| Cajamarca-Amazonas . . | 1955 | 3 | 22 | | 22 | Herrer y Morales (24). |
| *Totales*. . . . . | | *7* | *122* | *132* | *204* | |

*Nota*, — En algunos pacientes el diagnóstico se confirmó por más de un método.

## ASPECTO CLÍNICO

En el Perú no se ha efectuado estudio clínico exhaustivo en ninguno de los 204 pacientes en que se ha diagnosticado trypanosomiasis *cruzi*, debido a circunstancias especiales que analizaremos a continuación. Por este motivo no estamos en condiciones de establecer las características clínicas que adopta la enfermedad entre nosotros.

La casi totalidad de casos han sido hallazgos efectuados durante encuestas de carácter epidemiológico realizadas en población general no seleccionadas previamente (24, 26), y en pequeños grupos que si lo fueron teniéndose en cuenta sus antecedentes patológicos (signo de Romaña, etc.) (5) y las condiciones epidemiológicas de sus viviendas (5, 34).

En 197 enfermos, o sea en el 96,5 % de los casos, se efectuó el diagnóstico mediante la reacción de fijación del complemento o por la prueba del xenodiagnóstico, muchos días después de realizada la encuesta epidemiológica, cuando en la mayoría de las veces ya se había perdido contacto directo entre los investigadores y los grupos estudiados. En no pocas ocasiones el hallazgo de los pacientes se debió a la esforzada labor de distinguidos investigadores no médicos, o a la de estudiantes de Medicina que no tenían mucha experiencia clínica.

De los 204 pacientes estudiados, la mayoría fueron asintomáticos, algunos presentaron síntomas y signos de la fase aguda de la enfermedad y en cierto número no se consignó estudio clínico.

Con mayor frecuencia se ha observado la forma aguda de la enfermedad en niños menores de 5 años (2, 15, 34), pero también se la ha encontrado en jovenes de 17 y en adultos de 36, 39 y 40 años (5, 12, 27), habiéndose presentado tanto en hombres como en mujeres.

Los síntomas y signos de esta fase han sido consignados en la siguiente forma:

El complejo oftálmico-ganglionar se ha encontrado con frecuencia tanto en los niños como en los pocos adultos diagnosticados en la región sudoccidental del Perú (5, 15); no habiéndose presentado ni refirieron haberlo tenido, los enfermos de los departamentos de San Martin y Amazonas (24, 26). Dacrioadenitis se encontró con relativa frecuencia.

En un paciente con dos semanas de evolución se encontró un chagoma de inoculación en el miembro superior izquierdo. Lipochagomas genianos no han sido consignados en ninguno de los casos estudiados, debido posiblemente a que no fueron investigados.

La mayoría de casos agudos han evolucionado con fiebre poco elevada de tipo remitente o intermitente (5, 34, 39). En un niño estudiado por Uriel García (15) se comprobó fiebre continua de 13 días de duración, con ligeras remisiones matinales, y en el paciente de Lazo Taboada (27) la temperatura fué intermitente durante los primeros días, para evolucionar después en forma continua y con oscilaciones de 39 y 40,5°C.

En unos casos la fiebre estuvo precedida de escalofríos y seguida de sudores profusos que se repetían diaria o interdiariamente, mientras que en otros hubo escalofríos en forma bastante irregular.

Varios niños han tenido nauseas y vómitos y no pocos casos han referido cefalea y malestar general.

En numerosos pacientes se comprobó disnea, taquicardia, taquisfigmia, tonos cardíacos disminuídos de intensidad y en pocos soplo sistólico en el foco mitral y ritmo de galope (15). Dos niños de la casuística de Uriel García tuvieron síntomas y signos de miocarditis aguda, falleciendo uno de ellos con manifestaciones de insuficiencia cardíaca.

Edemas en miembros inferiores, anasarca, ascitis y derrames pleurales han sido referidos en varias historias clínicas; pero lo que con mayor frecuencia se ha consignado ha sido adenopatías periféricas generalizadas y principalmente compromiso de los ganglios preauriculares. También se ha encontrado frecuentemente esplenomegalia y en algunos casos hepatomegalia. Es importante hacer notar que muchos de los enfermos procedían de zonas donde la malaria es endémica.

Síntomas y signos de la fase crónica han sido consignados con menos frecuencia. Hay referencias en diversas encuestas epidemiológicas (24, 26) de casos que no presentaron síntomas ni signos de enfermedad en el momento de ser sometidos a las pruebas diagnósticas. Estos casos asintomáticos han sido observados tanto en hombres como en mujeres, en adultos como en menores; pero los referidos casos no pueden ser considerados como formas crónicas indeterminadas, debido a que no se efectuaron investigaciones clínicas ni electrocardiográficas para demostrar la ausencia de lesiones cardíacas.

Sabemos por informes recogidos en el valle de Majes, donde la trypanosomiasis *cruzi* es endémica, que el promedio de vida es relativamente corto y que es frecuente que los residentes del lugar mueran a los 50 años por «enfermedad al corazón».

## EL AGENTE TRASMISOR

El agente trasmisor de la enfermedad de Chagas es un artrópodo hematófago obligado, que pertenece a la familia *Reduviidae* y a la sub-familia *Triatominae*. De esta sub-familia, según la interesante monografía de Abalos y Wygodzinsky, hay 13 géneros en el continente americano que estan agrupados en 4 tribus: La tribu *Bolboderini*, que incluye los géneros *Belminus, Bolbodera* y *Parabelminus;* la tribu *Cavernicolini*, con el género *Cavernicola;* la tribu *Rhodniini*, de la que se ha encontrado en América los géneros *Psammolestes* y *Rhodnius;* finalmente la tribu *Triatomini*, que tiene 7 géneros en América: *Dipetalogaster, Eratyrus, Neotriatoma, Nesotriatoma, Panstrongylus, Paratriatoma* y *Triatoma*.

A esta lista de géneros de la sub-familia *Triatominae* señalados para América por Abalos y Wygodzinsky tenemos que agregar el género *Microtriatoma*, del que hemos encontrado una especie en la ciudad de Quincemil, del departamento del Cusco, el *Microtriatoma trinidadensis*.

En el Perú se han capturado especímenes del género *Belminus, Rhodnius, Triatoma, Panstrongylus* y *Microtriatoma*, habiéndose señalado la presencia de 10 especies [1], de las cuales 9 tienen diferente importancia epidemiológica en relación con la enfermedad de Chagas. Unas son conocidas por sus hábitos silvestres, siendo de especial interes porque mantienen el ciclo parasitario del *Trypanosoma cruzi* entre los mamíferos salvajes, siempre considerados como punto de partida de la infección trypanosómica en el hombre, cuando los tria-

[1] E. Dias (9), en 1952, mencionó que en el Perú se ha encontrado *R. robustus* en la región del río Ucayali y *R. pictipes* en Yurimaguas, pero nosotros no incluimos estas especies en la lista para el Perú debido a que no hemos encontrado los trabajos originales en la bibliografía que está a nuestro alcance.

tóminos silvestres ingresan en su vivienda, se adaptan, e infectan primero a los animales domésticos y después a él. Otras especies se caracterizan por sus acentuados hábitos domiciliarios, viviendo en contacto directo con el hombre y los animales domésticos, en el interior de las habitaciones. Estos triatóminos son responsables de la trasmisión de la trypanosomiasis americana.

En numerosos centros poblados de la zona endémica del Perú se ha verificado la presencia de casos agudos o crónicos de enfermedad de Chagas, animales domésticos naturalmente infectados por *Trypanosoma cruzi* y abundantes chirimachas con elevado índice de infección por *T. cruzi* en una misma vivienda (5).

El insecto trasmisor es conocido en América con diferentes denominaciones de carácter popular: así en Ecuador se le llama «chinchorro», «chinche sanguinófila»; en el Brasil, «barbeiro»; en Bolivia, «pito»; en el Uruguay, «chupon», y en la Argentina lo denominan «chinche gaucha», «chinche del monte». En el Perú el término «chirimacha» es el comunmente empleado para denominar a los triatóminos de la región sudoccidental y en el norte del país también lo llaman «chinchon».

Las especies de *Triatominae* encontradas en el Perú son las siguientes:

1. — *Triatoma infestans* Klug, 1834.

Esta especie fué descrita a base de ejemplares encontrados en Chile y se considera que ingresó al Perú procedente de la vecina república del sur (22).

El *Triatoma infestans* tiene actualmente amplia distribución en la región sudoccidental del Perú y por sus marcados hábitos domiciliarios se ha adaptado fácilmente a las características de las habitaciones humanas en las zonas urbanas, rurales y semi-rurales de los departamentos de Tacna (20, 41, 42), Moquegua (15, 20, 22, 42), Arequipa (4, 5, 6, 10, 11, 14, 16, 22, 31) e Ica (3). En diciembre de 1957 tuvimos ocasión de comprobar la presencia de esta especie en el interior de una casa moderna del distrito de Pueblo Libre, en la ciudad de Lima, donde posiblemente había ingresado, procedente de la casa

colindante en la cual reside una familia que con frequencia recibe encomiendas procedentes de Arequipa y Tacna.

Esta chirimacha ataca tanto al hombre como a los animales domésticos y se le ha encontrado con infección natural por *Trypanosoma cruzi* en los departamentos de Tacna (20), Moquegua (15, 19, 41, 42) y Arequipa (4, 5, 6, 10, 16, 31, 39).

El *Triatoma infestans* es el responsable de la transmisión de la enfermedad de Chagas en los departamentos del sur del Perú, al igual que en Argentina, Bolivia, Chile, Uruguay y algunas regiones del Brasil.

2. — *Triatoma carrioni* Larrousse, 1926.

Se le considera una especie autóctona de la república del Ecuador, donde el naturalista Clodoveo Carrión capturó por primera vez en 1924 ejemplares que posteriormente fueron empleados por Larrousse para describir esta nueva especie. Ha sido bien estudiada en esa república y se sabe que su área de dispersión alcanza la zona sur próxima a la frontera con el Perú, habiéndose estabelecido que las provincias de Loja y Ascuay son las más intensamente infestadas.

Recientemente se ha capturado especímenes de *T. carrioni* en los departamentos del norte del Perú, cerca de la frontera con Ecuador, y suponemos que ha ingresado a nuestro país procedente de la vecina república del norte.

Las investigaciones peruanas en relación con este triatómino son recientes y muy limitadas. Se le ha capturado en los departamentos de Piura (23) y Cajamarca (37, 38), en Huancabamba, Huinton, Aragoto, Montero, Querocotillo y Sallique. Especímenes procedentes de Sallique estaban naturalmente infectados por un trypanosoma con características morfológicas muy semejantes al *Trypanosoma cruzi* (38).

El *Triatoma carrioni* es de hábitos rurales e ingresa y se multiplica fácilmente en las habitaciones humanas construídas con materiales rústicos. No se le asigna importancia epidemiológica en la enfermedad de Chagas en el Ecuador y los resultados obtenidos en las pocas investigaciones realizadas entre nosotros no son suficientes para considerarlo trasmisor.

3. — *Triatoma dimidiata* Latreille, 1811.

Es una de las especies que tiene mayor zona de dispersión en América. Se caracteriza por sus acentuados hábitos domiciliarios, habiéndose adaptado a las características de la vivienda humana en las zonas urbanas y semi-rurales de numerosos países do Norte, Centro y Sud-América. Eduardo Del Ponte, en el año 1930, incluyó al Perú en la zona de dispersión de este triatómino.

El hombre y los animales domésticos son sus huéspedes preferidos y se le ha encontrado con infección natural por *T. cruzi* en Méjico, Honduras, Nicaragua, Guatemala, San Salvador, Panamá, Venezuela, Colombia y Ecuador.

El *Triatoma dimidiata* es el trasmisor de la enfermedad de Chagas de mayor importancia epidemiológica en Ecuador, donde se le considera especie estrictamente domiciliaria. Guayaquil es una de las ciudades más intensamente infestadas por esta chirimacha y allí se ha encontrado porcentajes de infección natural por *T. cruzi* que varían del 10 al 62 %.

En el Perú se ha capturado ejemplares de *T. dimidiata* en lugares poblados de la cuenca de los ríos Tumbes y Zarumilla (28), en el departamento de Tumbes, donde hay casas construídas de madera con techos de paja y hojas de plátano, que ofrecen magníficas condiciones favorables para las chirimachas. Yolanda Lizaraso examinó el contenido intestinal de 31 especímenes procedentes de Tumbes con resultado negativo para *Trypanosoma cruzi*.

Carecemos de información sobre el grado de diseminación que puede haber alcanzado este triatómino en nuestro país; pero teniendo en cuenta sus marcados hábitos domiciliarios y la facilidad con que se ha dispersado en numerosos países de América, que tienen condiciones climáticas semejantes con los departamentos del norte del Perú, es de suponer que pueda diseminarse y convertirse en elemento de importancia epidemiológica en la costa norte de nuestro territorio.

4. — *Panstrongylus geniculatus* Latreille, 1811.

Se sabe de la presencia de esta especie en el Perú desde el año 1811, en que Latreille la describió en especímenes procedentes de nuestro país, pero no se tuvieron nuevas informaciones hasta el

año 1948, en que apareció la importante publicación de Wygodzinsky (44), en la que informó haberlo identificado en muestras que procedían de Chanchamayo.

El *P. geniculatus* es de hábitos silvestres. Con frecuencia ha sido encontrado en cuevas de armadillos en la república Argentina y en pocas ocasiones se le ha capturado en el interior de viviendas humanas. Su zona de dispersión es bastante extensa en América. Se ha demostrado su infección natural por *Trypanosoma cruzi* en Argentina, Bolivia, Venezuela y Panamá.

Tenemos muy poca información sobre su distribución en el Perú y no ha sido posible demostrar infección natural por *T. cruzi* en los ejemplares encontrados en nuestro país.

5. — *Panstrongylus chinai* Del Ponte, 1929.

E. Del Ponte describió esta nueva especie (8) en un especímen capturado en el norte de nuestro país. Investigaciones posteriores efectuadas en Ecuador demostraron su amplia diseminación en las poblaciones de la frontera con el Perú, principalmente en la provincia de Loja.

El *Panstrongylus chinai* tiene amplia distribución en el norte del Perú y se le ha encontrado tanto en los centros poblados de la costa como en la región selvática, en lugares situados a nivel del mar y a 1500 m de altura. Herrer (23) señala que la zona de dispersión de este triatómino se extiende en la costa desde el valle de Fortaleza hasta Tumbes y que también lo ha capturado en el valle interandino del Marañon.

Wygodzinsky (44) redescribió el *P. chinai* en ejemplares procedentes de Balsas, Marañon. Ultimamente, Juana Arrarte (1) lo encontró en el pueblo de Sallique, en el departamento de Cajamarca. Nosotros lo hemos hallado en el departamento de La Libertad, durante una investigación entomológica realizada en el año 1953.

Se reproduce fácilmente en lugares de clima seco e intensamente caluroso, tiene hábitos domiciliarios y se ha adaptado a las características de las viviendas humanas en los lugares donde se ha señalado su presencia. En Perú y Ecuador ha sido capturado en el interior de viviendas y se sabe que ataca al hombre y a los animales domésticos.

Especímenes procedentes de Sallique estaban naturalmente infectados por *Trypanosoma cruzi* (1).

Según opinión de los que han estudiado los hábitos del *P. chinai* en el Perú, él no sería trasmisor importante en los departamentos del norte de nuestro país.

Creemos que nuevas y más amplias investigaciones epidemiológicas permitiran conocer mejor su grado de peligrosidad.

6. — *Panstrongylus herreri* Wygodzinsky, 1948.

Esta nueva especie solo ha sido encontrada en la región norte del Perú y hasta la fecha no se ha señalado su presencia en otro país de América. Fué descrita en 1948 por Wygodzinsky (44), a base de especímenes nacidos en el laboratorio, descendientes de ejemplares capturados en Bagua (23).

Tiene amplia distribución en los departamentos del norte, pues se le ha encontrado en los centros poblados de Bellavista, Pueblo Nuevo, Yamon, Lonya Grande, Roblepampa, Querocotillo, Jepelacio, Rioja y Moyobamba, en los departamentos de Amazonas, Cajamarca (23, 37) y San Martin (26, 34, 35, 36, 45), habiéndose demostrado que tenía infección natural por *Trypanosoma cruzi* en todos estos lugares.

El *Panstrongylus herreri* tiene hábitos domiciliarios y está adaptado perfectamente a las condiciones de la casa habitación, causando muchas molestias a los residentes de estos lugares, por sus frecuentes picaduras. Al igual que el *Triatoma infestans,* ataca al hombre y a los animales domésticos y se considera que es el responsable de la trasmisión de la enfermedad de Chagas en el norte del Perú. Se ha observado que los especímenes de *P. herreri* capturados en los departamentos del norte presentan índices de infección natural por *T. cruzi* tan elevados como los encontrados en ejemplares de *Triatoma infestans* procedentes del sur (ver cuadro n.° 2).

7. — *Panstrongylus rufotuberculatus* Champion, 1899.

Recientemente se ha encontrado esta especie en centros poblados de la cuenca del río Zarumilla, en el departamento de Tumbes (28),

y no hay referencias de que haya sido capturada en otros lugares de nuestro país. Se sabe que su zona de distribución es amplia en la vecina república del Ecuador, donde las provincias de El Oro y Loja son las más intensamente infestadas.

El *P. rufotuberculatus* posee habitos domiciliarios y está adaptado a las condiciones de la vivienda humana, donde vive en contacto directo con el hombre. En Venezuela y Ecuador ataca a éste y a los animales domésticos, en las casas habitación de las zonas rurales y semi-rurales. En los referidos países se le ha encontrado con infección natural por *T. cruzi*.

Hay referencias de que en el Perú también ataca al hombre y a los animales domésticos. Se le ha capturado en el interior de casas de madera, con techos de paja y hojas de plátano, que reunen condiciones muy favorables para estas chirimachas (28).

Desconocemos su importancia epidemiológica entre nosotros, pues aún no se ha estudiado bien sus hábitos en la zona de su distribución, ni fué posible examinar el contenido intestinal de los especímenes capturados en Tumbes.

8. — *Belminus peruvianus* Herrer, Lent y Wygodzinsky, 1954.

Solamente se han descrito 3 especies del género *Belminus* y de éstas el *B. peruvianus* ha sido últimamente descrito a base de ejemplares recolectados en la cuenca del Marañon (18, 24). No se ha encontrado este triatómino en otros países de América.

El *B. peruvianus* tiene hábitos domiciliarios y, al igual que muchas otras chirimachas, se ha adaptado en la vivienda humana de los centros poblados de la cuenca del Marañon, pues siempre se le ha capturado en el interior de las habitaciones humanas y en los mismos lugares en que se encuentra el *P. herreri*. No se ha examinado el contenido intestinal de los especímenes capturados en los departamentos del norte y, en opinión de los que han estudiado sus hábitos, parece no tener rol de importancia en la trasmisión de la enfermedad de Chagas.

9. — *Rhodnius pallescens* Barber, 1932.

Fué encontrado durante las investigaciones efectuadas por nosotros en el departamento de Madre de Dios (7), con el fin de estudiar la patología tropical de esa región.

El *Rhodnius pallescens* es de hábitos domiciliarios en Panamá. donde se ha capturado especímenes que tenían infección natural por *T. cruzi* en el interior de habitaciones humanas y se ha demostrado su presencia en las casas de una docena de centros poblados.

En nuestro país se han capturado ejemplares en viviendas humanas de la ciudad de Puerto Maldonado, en el departamento de Madre de Dios, comprobándose su infección natural por *T. cruzi* (7). Aun no tenemos suficiente información sobre su importancia epidemiológica entre nosotros.

10. — *Microtriatoma trinidadensis* (Lent).

Durante las investigaciones efectuadas por nosotros en la ciudad de Quincemil, del departamento del Cusco, en 1957, se capturó en el interior de una casa habitación un ejemplar de *Microtriatoma trinidadensis*. Este especímen fué remitido, con otro de *Rhodnius pallescens*, al Dr. Reece I. Sailer de la Sección de Entomología del Departamento de Agricultura de los Estados Unidos de Norteamérica, quien tuvo la gentileza de identificarlos. A su solicitud ha sido donado para la colección del U.S. National Museum.

No fué posible examinar las heces de este triatómino y no sabemos si tiene importancia epidemiológica en relación con la enfermedad de Chagas.

Es la primera vez que se le encuentra en territorio peruano y en la interesante monografía de Abalos y Wygodzinsky (1951) no figura en la lista de géneros de la sub-familia *Triatominae* encontrados en América.

En numerosas encuestas epidemiológicas efectuadas entre los años 1944 y 1957, en los departamentos del sur y del norte del Perú, se han encontrado elevados porcentajes de chirimachas, con infección natural por *Trypanosoma cruzi*, en zonas urbanas, rurales y semi-rurales. Los resultados de estas encuestas figuran en el cuadro n.° 2, en

el que se puede apreciar los porcentajes de infección en los triatóminos capturados en los diferentes lugares del territorio peruano.

Abilia Ticse (43) realizó en 1951 interesantes observaciones experimentales sobre el ciclo evolutivo del *Triatoma infestans* y Yolanda Lizaraso (29), en 1957, estudió las características morfológicas de los huevos de la mayoría de especies de triatóminos que hay en el Perú, a fin de poder identificar éstos.

A semejanza de lo observado por Pellegrino en huevos de *T. infestans* y de *P. megistus* en el Brasil, Lumbreras y colaboradores (35) encontraron que los huevos de *Panstrongylus herreri* estaban naturalmente parasitados por el microheminóptero *Telenomus fariae*.

El *Triatoma infestans* y el *Panstrongylus herreri* se han adaptado perfectamente a las condiciones y características de la vivienda urbana, rural y semi-rural, que predomina en el Perú. En algunos lugares, como en el valle de Majes (5) y en la cuenca del Marañon (23), es dificil encontrar una casa habitación donde no sea posible capturarlos en los sitios donde habitualmente se esconden. Se les halla en huecos y resquebrajaduras de las paredes deficientemente revocadas, detrás de los cuadros, de los almanaques y de las figuras de papel con que las gentes acostumbran adornar las paredes de sus dormitorios, así como en los techos de caña, detrás de los baúles, muebles y cajones que no se remueven diariamente, lo mismo que en las prendas de vestir, en los dobleces de las cortinas empleadas para dividir las habitaciones y en la cara inferior de los catres. En los cuyeros se les captura en número considerable, bastando remover los adobes o piedras que se usan para su construcción.

Hay triatóminos en lugares situados a nivel del mar (3, 6, 19) y a 3.200 m de altura (19); pero las zonas comprendidas entre 600 m y 1.500 m presentan mayores índices de infestación (19).

Desconocemos cual es la variación estacional de la población de las diferentes especies en el Perú. Por informes obtenidos en diferentes zonas en que se han efectuado investigaciones epidemiológicas, sabemos que es posible capturarlas durante todo el año y que en los meses de verano se aprecia un notable incremento de ellas. Los pobladores de las zonas endémicas refieren además, en los meses de mayor calor, las chirimachas pican con mayor frecuencia.

Con referencia a sus hábitos alimenticios, el *Triatoma infestans* y el *Panstrongylus herreri* atacan al hombre y a los animales domés-

CUADRO N.º 2 — *Infección natural por* Trypanosoma cruzi *en triatóminos capturados en diferentes lugares del Perú entre los años 1944 y 1957*

| Departamento | Lugar | Año | Espécie de triatómino | Número de examinados | Porcentaje de infecc. | Autor |
|---|---|---|---|---|---|---|
| Tacna . . . . | Tarata | 1955 | *T. infestans* | 58 | 67,24 | F. y N. Náquira (41). |
| Tacna . . . . | Caplina | | | | | |
| Moquegua . . | Sama | 1955 | *T. infestans* | 1.354 | 35,45 | A. Herrer (19). |
| Arequipa . . . | Locumba | | | | | |
| | Moquegua | | | | | |
| | Arequipa | | | | | |
| | Ocoña | | | | | |
| Moquegua . . | Para Chico | 1957 | *T infestans* | 221 | 31,6 | F. y N. Náquira (42). |
| Tacna . . . . | Para Grande | | | | | |
| | Calientes | | | | | |
| | Challata | | | | | |
| | Tarata | | | | | |
| | Locumba | | | | | |
| | Samengua | | | | | |
| Moquegua . . | Moquegua | 1951 | *T. infestans* | 288 | 66,6 | U. García (15). |
| | Santa Rosa | | | | | |
| | Torata | | | | | |
| | Tumilca | | | | | |
| | Samengua | | | | | |
| Arequipa . . . | Vitor | 1944 | *T. infestans* | 607 | 36,2 | A. Herrer (17). |
| | Quishuarani | | | | | V. M. Ayulo. |
| | Sihuas | | | | | |
| | Majes | 1950 | *T. infestans* | 419 | 42 | Cornejo Donayre. |
| | Majes | 1951 | *T. infestans* | 506 | 34,38 | Lumbreras C. (4, 5). |
| | Camana | 1951 | *T. infestans* | 129 | 52,71 | Cornejo Donayre y Lumbreras C. (6). |
| | Arequipa | 1952 | *T. infestans* | 134 | 34,32 | Lumbreras C. (31). |
| | Sihuas | 1957 | *T. infestans* | 837 | 34,09 | J. Montesinos, C. Náquira y otros (39). |
| Ica. . . . . | Ica | 1946 | *T. infestans* | 206 | 0 | V. M. Ayulo (3). |
| | Nazca | | | | | |
| Cajamarca . . | Cutervo | 1955 | *P. herreri* | 31 | 35,48 | Lumbreras, Estrada, Arrarte y Tejada (37). |
| | Querocotillo | | *T. carrioni* | 6 | 0 | |
| Cajamarca . . | Cumba | 1955 | *P. herreri* | 73 | 45,2 | A. Herrer (23). |
| Amazonas . . | Cajaruru | | | | | |
| | Jaen | | | | | |
| | Pueblo Nuevo | | | | | |
| | Yamon | | | | | |
| | Lonya Grande | | | | | |
| | Roblepampa | | | | | |
| | Bellavista | | | | | |
| San Martin . . | Moyobamba | 1955 | *P. herreri* | 85 | 16,86 | Lumbreras, Arrarte y Guevara (33). |
| | Rioja | | | | | |
| | Moyobamba | 1955 | *P. herreri* | 649 | 24,65 | Lumbreras, Arrarte, Guevara y Sipan (36). |
| | Rioja | | | | | |
| | Jepelacio | 1955 | *P. herreri* | 253 | 7,1 | Zapatel y Acha (45). |
| | Moyobamba | 1956 | *P. herreri* | 296 | 31,4 | A. Herrer (26). |
| Tumbes. . . . | Cuenca | 1955 | *T. dimidiata* | 31 | 0 | Y. Lizaraso (29). |
| | Tumbes y Zarumilla | | | | | |

ticos que se crian en el interior de las habitaciones o en lugares próximos a ellas. Pican durante la noche y prefieren hacerlo en la cara o en otras partes descubiertas del cuerpo, durando la succión de 5 a 15 minutos. No producen dolor; pero posteriormente aparece en la zona de la picadura una pápula de regular tamaño que produce prurito intenso.

## EL AGENTE ETIOLÓGICO

El agente etiológico de la enfermedad de Chagas fué encontrado por primera vez en el Perú el año 1917 (10), en el contenido intestinal de un *Triatoma infestans,* capturado en un valle del departamento de Arequipa. Posteriormente, Escomel (12, 13) comunicó el hallazgo del primer caso de enfermedad de Chagas entre nosotros y describió las características morfológicas del *T. cruzi,* encontrado en la sangre periférica de un paciente que procedía del departamento de Madre de Dios. En esta descripción señaló que el trypanosoma tenía una longitud de 20 $\mu$ a 40 $\mu$ y un blefaroblasto puntiforme y como tales datos no correspondían a la descripción clásica de este parásito, Yorke lo consideró como una nueva especie a la que denominó *T. escomeli.*

Ultimamente, E. Dias (9) ha sugerido que el *T. escomeli* y el *T. rangeli* son iguales, es decir se trataría de la misma especie, creando cierta duda y, como anota Zeledon (46), estableciendo un problema de prioridad.

Nosotros hemos aislado en cultivo puro una cepa de *T. cruzi* del contenido intestinal de *Rhodnius pallescens,* capturado en la ciudad de Puerto Maldonado, en el departamento de Madre de Dios (7). Esto nos pone en condiciones de afirmar que el agente etiológico de la enfermedad de Chagas se encuentra en ese departamento, infectando en condiciones naturales a triatóminos y mamíferos, pero no podemos resolver el problema a que ha dado lugar la descripción de Escomel.

Recientes investigaciones epidemiológicas han permitido estudiar varias cepas de *Trypanosoma cruzi* (1, 7, 17, 23, 25, 33, 37, 39, 40), procedentes de diferentes zonas del territorio nacional, y las inoculaciones en ratones y ratas blancas, de 2 a 3 semanas de nacidas, demuestran que son diferentes patogenicidad y virulencia (7, 25, 40).

Las observaciones clínicas efectuadas en la cuenca del Marañon (19, 24) en el valle de Majes (5), en Tacna (41, 42) y en Moquegua (15, 41) establecen que los síntomas y signos de la trypanosomiasis *cruzi* que se observan en Arequipa, Tacna y Moquegua no se presentan frecuentemente en los pacientes que contraen la enfermedad en la cuenca del Marañon.

Es necesario realizar nuevas y más amplias investigaciones a fin de aclarar las observaciones preliminares anotadas, tanto desde el punto de vista clínico como desde el punto de vista experimental.

## RESERVORIO

Al igual que en Bolivia, en el Perú también constituye el cuy o cobayo el reservorio más importante de la enfermedad de Chagas. En casi todas las encuestas epidemiológicas realizadas en el país se ha demostrado que el cobayo se encuentra infectado por *T. cruzi* en condiciones naturales.

Ayulo y Herrer (2) demostraron infección por *T. cruzi* en 25 de 443 cuyes examinados en Vítor, Sihuas, Quishuarani y Arequipa. U. García (15), en 1951, obtuvo 2 cobayos infectados de 15 examinados en Moquegua. Nosotros (5) verificámos la infección en 2 cobayos de un total de 21 sometidos a examen directo de la sangre, durante la encuesta de 1951, en Majes. Herrer (19), en Tacna y Moquegua, de 297 encontró 17 infectados por *T. cruzi*. Los hermanos Náquira (41) obtuvieron un xenodiagnóstico positivo de un total de cuatro aplicados a igual número de cuyes en Tacna y finalmente, en 1957 (42), los mismos autores encontraron un xenodiagnóstico positivo en un cobayo también de Tacna.

Se ha observado que el cobayo es uno de los animales que con mayor frecuencia se cría en el interior de las habitaciones humanas (19, 21). Montesinos y colaboradores (39) demostraron que este animal se criaba en el 83,33 % de 180 viviendas estudiadas de Sihuas. Nosotros encontrámos en Majes (5) que en el 65,28 % de las viviendas estudiadas se criaba cobayos en el interior de las casas, generalmente en lugares muy próximos al dormitorio y muchas veces en esta misma habitación.

Tan lamentable costumbre, muy generalizada en el Perú y en Bolivia, posiblemente se deba al bajo costo de estos animales y a su

facil crianza y reproducción, siendo pues los que se encuentran en mayor número entre los animales que viven en promiscuidad con los residentes de las zonas urbanas, rurales y semi-rurales de la zona endemica del Perú (21). Además, las encuestas epidemiológicas han revelado que los cuyeros, o criaderos de cuyes, constituyen los mejotes criaderos de chirimachas (4, 5, 15, 16, 19, 21), debido a que ofrecen condiciones favorables de humedad, oscuridad y albergue, con sus numerosas grietas y resquebrajaduras.

Los triatóminos capturados en criaderos de cobayos arrojaron los mayores porcentajes de infección por *T. cruzi* en las encuestas de 1944, con 73,5 % (16); de 1950, con 40 % (4); de 1951, con 51,42 % (5), y de 1955, con 50,9 % (21).

El hombre es también reservorio importante de la enfermedad de Chagas, debido que hasta ahora se carece de tratamiento eficaz y las personas infectadas permanentemente ofrecen ocasión para que las chirimachas, a su vez, puedan infectarse.

Se ha encontrado, asi mismo, infección natural en perros (2, 15, 26) y en gatos (19); pero a estos animales se les asigna menor importancia como reservorios.

## OTROS FACTORES EPIDEMIOLÓGICOS

*Vivienda.* — Representa ésta el factor epidemiológico de mayor importancia, pudiendo asegurarse que es raro encontrar la enfermedad en lugares donde ella es higiénica y construída con materiales nobles y con paredes y techos bien revocados. En las zonas endemicas del Perú predomina el tipo de construcción con material de adobe o paredes de quincha, mal revocadas con yeso o con barro (4, 5, 6, 16, 31, 39, 41) y techos de madera o de caña con las consiguientes resquebrajaduras y grietas, que sirven de criaderos a las chirimachas. En la región selvática abundan las viviendas con paredes de poma, caña o madera y con techos de hojas de palmera (7, 36), que permiten el fácil ingreso y permanencia de los triatóminos.

En las diversas encuestas epidemiológicas realizadas entre nosotros se ha comprobado que las casas visitadas estaban infestadas por chirimachas en el 11,1 % y 39 % (19) y el 53,18 % (36), para citar solo unos ejemplos. En numerosos pueblos estas viviendas estan sobrepobladas, siendo frecuente que familias de siete y ocho miem-

bros ocupen casas de una, dos o tres habitaciones y que en las casas de una habitación, esta sirve de dormitorio, cocina y corral. En el valle de Majes hemos encontrado un promedio de 2,08 personas por cama (5). Lumbreras halló un 2,38 en la urbanización de Miraflores, en Arequipa, y Montesinos y colaboradores (39) 2,16 en el valle de Sihuas. Ultimamente nosotros hemos encontrado 2,7 personas por cama como promedio en el departamento de Madre de Dios (7).

## DISEMINADORES DE LA ENFERMEDAD DE CHAGAS

La trypanosomiasis *cruzi* se va extendiendo lenta pero constantemente en el territorio peruano y esto se debe, entre otros factores, a la acción diseminadora que realiza el hombre al trasportar chirimachas de lugares infestados a otros que no lo estan, pero que reunen condiciones favorables para la permanencia y multiplicación de estos insectos en el interior de las viviendas. Es bien sabido que los triatóminos no se trasladan a grandes distancias, valiéndose de sus propios medios de locomoción, si no que escondidos en paquetes, encomiendas y carga en general son transportados por vehículos motorizados o por arrieros, pudiendo así realizar viajes de larga duración sin que les afecte un ayuno prolongado (22).

Se considera que el *Triatoma infestans* ingresó en el Perú, procedente de Chile, entre los años 1879 y 1883 (22) y se ha extendido, pasando por Tacna, Moquegua y Arequipa, hasta llegar a la ciudad de Ica, en donde fué señalada su presencia en el año 1946 (3). Actualmente ha llegado a la ciudad de Lima, donde lo hemos encontrado en una casa de Pueblo Libre, a la que habría pasado de la casa vecina, donde reside una familia que frecuentemente recibe encomiendas procedentes de Arequipa y Lima.

Como señala Herrer (22), el hombre es el principal diseminador de chirimachas, por lo general inconscientemente, pero a veces en forma deliberada. Sabemos de alguien que las puso en libertad «para que hagan cría».

Al igual que la diseminación del insecto transmisor, el trasporte de cobayos infectados con *T. cruzi* de regiones endémicas a zonas no infectadas contribuye a que la enfermedad se extienda, siendo el hombre quien también realiza esta inconveniente labor.

## EDUCACIÓN SANITARIA

Lamentablemente, aun no se ha realizado entre nosotros una campaña de educación sanitaria en relación con la enfermedad de Chagas y los residentes de las zonas endémicas ignoran el peligro a que están expuestos y los medios de que pueden valerse para evitar la enfermedad. Esta ignorancia unida a la miseria en que viven, las características de la vivienda y su sobre-población, la promiscuidad con animales domésticos, que sirven de reservorio al *T. cruzi,* y la abundancia de chirimachas han creado todos los eslabones necesarios de la cadena epidemiológica de esta enfermedad en numerosos y extensos lugares de nuestro territorio.

## PROFILAXIS

En el Perú ya se han realizado algunas campañas profilácticas y actualmente se tiene la certidumbre que sería posible erradicar el *Triatoma infestans* de todos los departamentos del sur, mediante el empleo del gamexano y otros insecticidas de acción residual. Cuando se amplien los conocimientos sobre la trypanosomiasis americana en el norte del país, se determinarán los insecticidas más convenientes.

Es necesario mejorar las condiciones de la vivienda rural y semi-rural y sería de mucho provecho una legislación que tienda a evitar la edificación de casas sin que medie la autorización del gobierno local o central, a fin de que las nuevas construcciones en las zonas endémicas se realicen de acuerdo con directivas encaminadas a evitar que puedan convertirse en criaderos de triatóminos. En Argentina la campaña profiláctica de la enfermedad de Chagas, basada en las mejoras de la casa habitación, dió magníficos resultados.

## IMPORTANCIA MÉDICO-SOCIAL

En la Primera Reunión Panamericana de Mesa Redonda sobre Enfermedad de Chagas, convocada por la Oficina Sanitaria Panamericana, que se efectuó en Tucuman, Salta y Jujuy, durante el mes de julio de 1949, se estableció que esta trypanosomiasis, en su forma aguda y crónica, tiene importancia como causa de muerte y como

factor que disminuye la capacidad de trabajo en las regiones rurales y semi-rurales de América Central y del Sur.

Distinguidos investigadores nacionales han contribuído al mejor conocimiento y a la profilaxis de esta endemia. El camino recorrido debe servir de base para que las nuevas generaciones médicas contribuyan a su erradicación. Pensamos que las Cátedras de Parasitología y Medicina Tropical de las nuevas Facultades de Medicina no omitirán esfuerzo para continuar las investigaciones sobre este aspecto de la patología nacional y comunicarán sus resultados al Ministerio de Salud Pública para que este efectúe las campañas profilácticas.

## RESUMEN Y CONCLUSIONES

1. La enfermedad de Chagas está ampliamente diseminada en el territorio peruano, habiéndose demostrado su presencia en los departamentos de Tacna, Moquegua, Arequipa, Madre de Dios, Amazonas y San Martin.

2. En el Perú se ha diagnosticado un total de 204 casos de trypanosomiasis *cruzi* hasta el año 1957, de los cuales 202 corresponden a los últimos siete años, debido al mayor número de encuestas epidemiológicas realizadas en este lapso.

3. Se presenta un resumen de los síntomas y signos observados en los pacientes de trypanosomiasis *cruzi* diagnosticados en el Perú, señalándose que el mayor número de casos agudos se observó en niños menores de 5 años y los casos crónicos tanto en adultos como en menores.

4. Se ha capturado triatóminos capaces de trasmitir la enfermedad de Chagas en los departamentos de Tacna, Moquegua, Arequipa, Ica, Lima, Junin, Cusco, Madre de Dios, La Libertad, Piura, Tumbes, Cajamarca, Amazonas y San Martin.

5. En el Perú se han encontrado las siguientes especies de triatóminos: *Triatoma infestans, Triatoma dimidiata, Triatoma carrioni, Panstrongylus herreri, Panstrongylus geniculatus, P. chinai, Panstrongylus rufotuberculatus, Belminus peruvianus, Rhodnius pallescens* y *Microtriatoma trinidadensis*. El *T. infestans* es el unico transmisor en la región sudoccidental y el *P. herreri* es el transmisor más importante en el norte del país.

El *T. infestans* ingresó en el Perú procedente de Chile y las especies *T. dimidiata, T. carrioni* y *P. rufotuberculatus* lo hicieron posiblemente desde la vecina república del Ecuador.

6. El reservorio de la trypanosomiasis *cruzi* más importante en el Perú es el cuy o cobayo; pero también se ha encontrado infección natural en perros y gatos de la zona endémica.

7. Las condiciones y características de la vivienda humana en las zonas urbanas, rurales y semi-rurales de la zona endémica del Perú constituyen factor importante en la epidemiología de la enfermedad de Chagas, pues se ha demostrado que los triatóminos ingresan y se multiplican fácilmente en el interior de las habitaciones.

El hombre vive en marcada promiscuidad con animales domésticos que sirven de reservorio al *T. cruzi* y la vivienda está sobrepoblada.

8. El hombre actúa como diseminador de la enfermedad de Chagas al trasportar triatóminos, cobayos, perros y gatos con infección natural por *T. cruzi*.

## SUMMARY AND CONCLUSIONS

1. Chagas' disease is widely diseminated in the Peruvian territory, having been demonstrated its existence in the departments of Tacna, Moquegua, Arequipa, Madre de Dios, Amazonas and San Martin.

2. Up to 1957, 204 cases of trypanosomiasis *cruzi* have been diagnosticated, of which 202 belong to the last 7 years, owing to the major number of epidemiological surveys which took place in this lapse of time.

3. A summary of the symptoms and signals observed in patients with trypanosomiasis *cruzi* diagnosticated in Peru is submitted. It is noted in it that the majority of acute cases were observed in children under 5 years of age and chronic cases in adults as well as in youngsters.

4. Triatominos capable of transmitting Chagas' disease were captured in the departments of Tacna, Moquegua, Arequipa, Ica, Lima, Junin, Cuzco, Madre de Dios, La Libertad, Piura, Tumbes, Cajamarca, Amazonas and San Martin.

5. The following species of triatominos have been found in Peru: *Triatoma infestans, Triatoma dimidiata, Triatoma carrioni, Panstrongylus herreri, Panstrongylus geniculatus, P. chinai, Panstrongylus rufotuberculatus, Belminus peruvianus, Rhodnius pallescens* and *Microtriatoma trinidadensis*. *T. infestans* is the only vector important in the north of the country.

*T. infestans* entered Peru from Chile and species *T. dimidiata, T. carrioni* and *P. rufotuberculatus* possibly came from our neighbour republic of Ecuador.

6. The most important reservoir of trypanosomiasis *cruzi* in Peru is the guinea pig, but also cats and dogs have been found to be naturally infected in the endemic zone.

7. The conditions and characteristics of the human dwellings in the urban zone, of the rural and semi-rural of the endemic zone of Peru, constitute the most important factor in the epidemiology of Chagas' disease, since it has been demonstrated that the triatominos enter and reproduce itself easily in the interior of the rooms. Man lives in a marked promiscuity with domestic animals which serve as reservoirs of *T. cruzi*, and the dwelling is overpopulated.

8. Man acts as disseminator of Chagas' disease transporting triatominos, guinea pigs, dogs and cats naturally infected by *T. cruzi*.

## BIBLIOGRAFÍA

1. Arrarte, Juana — «Nota preliminar acerca de la infección del *Panstrongylus chinai* (Del Ponte, 1929) por trypanosoma *cruzi*», *Rev. Mé. Per.*, Lima, **26**: 447-448, 1955.

COLOMBIA
ECUADOR
TUMBES
PIURA
AMAZONAS
LORETO
LAMBAYEQUE
SAN MARTIN
CAJAMARCA
BRASIL
N
LA LIBERTAD
ANCASH
HUANUCO
PASCO
JUNIN
LIMA
MADRE DE DIOS
OCEANO
PACIFICO
HUANCAVELICA
CUSCO
APURIMAC
ICA
AYACUCHO
PUNO
BOLIVIA
AREQUIPA
MOQUEGUA
TACNA
CHILE
0 100 200 300 400 Km
ESCALA
ENFERMEDAD DE CHAGAS EN EL PERU
Enfermos de Tripanosomiasis americana 0
Triatóminos infectados en condiciones naturales por Tripanosoma cruzi
Triatóminos no infectados en condiciones naturales por Tripanosoma cruzi.
Animales domésticos infectados en condiciones naturales por Tripanosoma cruzi.
Cornejo Donayre

ECUADOR
COLOMBIA
BRASIL
N
OCEANO
PACIFICO
BOLIVIA
CHILE
TRIATOMINOS ENCONTRADOS EN EL PERU
Triatoma infestans
Triatoma carrioni
Triatoma dimidiata
Ponstrongylus herreri
Ponstrongylus chinai
Ponstrongylus geniculatus
Ponstrongylus rufotuberculatus
Rhodnius pallescens
Belminus peruvianus
Microtriatoma trinidadensis
0 100 200 300 400 Km
ESCALA
Cornejo Donayre

2. Ayulo, V. M., y Herrer, A. — «Estudios sobre trypanosomiasis en el Perú. I. Observaciones en el departamento de Arequipa», *Rev. Med. Exp.*, Lima, **3**: 96-117, 1944.
3. Ayulo, V. M. — «Estudios sobre trypanosomiasis americana en el Perú. Observaciones en el departamento de Ica», *Rev. Med. Exp.*, Lima, **5**: 21-35, 1946.
4. Cornejo Donayre, A., y Lumbreras Cruz, H. — «Estudios preliminares sobre epidemiología de la enfermedad de Chagas en el valle de Majes», *Arch. Per. Patol. Clin.*, Lima, **4**: 121-130, 1950.
5. Cornejo Donayre, A., y Lumbreras Cruz, H. — «Encuesta epidemiológica sobre enfermedad de Chagas en el valle de Majes. Presentación de diez nuevos casos», *Arch. Per. Patol. Clin.*, Lima, **5**: 79-106, 1951.
6. Cornejo Donayre, A., y Lumbreras Cruz, H. — «Estudios preliminares sobre epidemiología de la enfermedad de Chagas en el valle de Camana», *Arch. Per. Patol. Clin.*, Lima, **5**: 107-112, 1951.
7. Cornejo Donayre, A. — *Investigaciones sobre Patología Tropical en el departamento de Madre de Dios. I. Enfermedad de Chagas.* En prensa. 1958.
8. Del Ponte, E. — «Algunas especies nuevas del género *Triatoma*», *Bol. Soc. Ent.*, Buenos Aires, **1** (4): 3-8, 1929.
9. Dias, Emmanuel — «Doença de Chagas nas Américas. V. Equador e Peru», *Rev. brasil. Malar. D. Trop.*, **4** (4): 319-325, 1952. Citado por 46.
10. Escomel, Edmundo — «Insectos hemípteros, heterópteros, hematófagos en nuestros valles del sur del Perú. Encierran formas protozoaicas, herpetomónicas. Existe entre nosotros la enfermedad de Chagas?», *La Ref. Méd.*, Lima, **3**: 121-122, 1917.
11. Escomel, Edmundo — «A propósito de los insectos hemípteros, heterópteros y hematófagos en nuestros valles del sur», *La Ref. Méd.*, Lima, **4**: 6, 1918.
12. Escomel, Edmundo — «La trypanosomiase humaine existe dans les forts orientaux du Perou», *Bull. Soc. Pat. Exot.*, Paris, **12**: 723-726, 1919.
13. Escomel, Edmundo — «Comprobación clínica y microscópica de la existencia en el Perú de la trypanosomiasis americana», *An. Fac. Med.*, Lima, **5**: 14-17, 1920.
14. Escomel, Edmundo — *El* Triatoma infestans *en algunos valles del Perú (chirimachas).* Obras Científicas. Ed. Torres Aguirre, pp. 775-780, 1929.
15. García Caceres, U. — «Contribución al estudio de la patología endémica de los valles del extremo sur de la costa peruana. La enfermedad de Chagas en el valle de Moquegua», *Rev. Med. Exp.*, Lima, **8**: 227-243, 1951.
16. Herrer, A., y Ayulo, V. M. — «Estudios sobre trypanosomiasis americana en el Perú. II. Observaciones entomológicas», *Rev. Med. Exp.*, Lima, **3**: 118-133, 1944.
17. Herrer, A. — «Nota preliminar sobre enfermedad de Chagas en la cuenca del río Marañon», *Rev. Méd. Per.*, Lima, **25**: 356-358, 1954.
18. Herrer, A., Lent, H., y Wygodzinsky, P. — «Contribución al conocimiento del género *Belminus* Stal, 1859 *(Triatominae, Reduviidae, Hemiptera)*», *An. Inst. Med. Reg.*, Tucuman, **4** (1): 85-106, 1954.

19. Herrer, A. — «Trypanosomiasis americana en el Perú. I. El insecto vector y los animales que actúan de reservorio de la enfermedad de Chagas en la región sudoccidental», *Rev. Med. Exp.*, Lima, **9**: 23-37, 1955.
20. Herrer, A. — Trypanosomiasis americana en el Perú. II. Repercusión del uso del DDT en la incidencia de la enfermedad de Chagas en algunos valles de la región sudoccidental», *Rev. Med. Exp.*, Lima, **9**: 38-43, 1955.
21. Herrer, A. — «Trypanosomiasis americana en el Perú. III. Importancia del cobayo como reservorio de la enfermedad de Chagas en la región sudoccidental», *Rev. Med. Exp.*, Lima, **9**: 45-57, 1955.
22. Herrer, A. — «Trypanosomiasis americana en el Perú. IV. Ingreso del *Triatoma infestans* al territorio peruano, su dispersión en éste y posibilidades de ser erradicado», *Rev. Med. Exp.*, **9**: 57-67, 1955.
23. Herrer, A. — «Trypanosomiasis americana en el Perú. V. Triatóminos del valle interandino del Marañon», *Rev. Med. Exp.*, Lima, **9**: 69-77, 1955.
24. Herrer, A., y Morales, J. — «Trypanosomiasis americana en el Perú. VI. Verificación de la enfermedad de Chagas en la cuenca del Marañon», *Rev. Med. Exp.*, Lima, **9**: 83-91, 1955.
25. Herrer, A., y Diaz, J. — «Trypanosomiasis americana en el Perú. VII. Cepas de *Trypanosoma cruzi* de escasa virulencia», *Rev. Med. Exp.*, **9**: 92-101, 1955.
26. Herrer, A. —«Observaciones sobre enfermedad de Chagas en la provincia de Moyobamba (departamento de San Martin)», *Rev. Med. Exp.*, Lima, **10**: 59-73, 1956.
27. Lazo Taboada, F., y García-Durand, L. — «Un caso de enfermedad de Chagas en el departamento de Arequipa», *Rev. Méd. Arequipeña*, **4**: 4-7, 1953.
28. Lizaraso, Yolanda — «Nota sobre el hallazgo de *Triatoma dimidiata* y *Panstrongylus rufotuberculatus* en el Perú», *Rev. Med. Exp.*, Lima, **9**: 119-121, 1955.
29. Lizaraso, Yolanda — *Estudio morfológico de los huevos de algunos triatóminos hallados en el Perú.* Tesis de Bachiller. Fac. Ciencias. Universidad Nacional Mayor de San Marcos, Lima, 1957.
30. Liceras Zarate, J. — *Estudio morfológico comparativo de las cepas peruanas «marañon», y «costa» del* Trypanosoma cruzi *Chagas, 1909, en el periodo hemático.* Tesis de Bachiller. Fac. Ciencias, Universidad Nacional Mayor de San Marcos, Lima.
31. Lumbreras, H. — «Epidemiología de la enfermedad de Chagas en la urbanización de Miraflores de Arequipa», *Arch. Per. Pat. Clín.*, Lima, **4**: 191-200, 1952.
32. Lumbreras, H. — «Un método de registro epidemiológico aplicado al diagnóstico y control de la enfermedad de Chagas», *Arch. Per. Pat. Clín.*, Lima, **8**: 115-124, 1954.
33. Lumbreras, H., Arrarte, Juana, y Guevara, Brígida — «La presencia del *Panstrongylus herreri* Wygodzinsky, 1948, y su infección por el *Schizotrypanum cruzi* en San Martin», *Rev. Méd. Per.*, Lima, **26**: 11-13, 1955.

34. Lumbreras, H., Arrarte, Juana, y Guevara, Brígida — «Primeros casos de enfermedad de Chagas en el departamento de San Martin», *Rev. Méd. Per.*, Lima, **26**: 42-47, 1955.

35. Lumbreras, H., Arrarte, Juana, y Guevara, Brígida — «Control biológico de los vectores de la enfermedad de Chagas. La presencia del *Telenomus fariae* infestando huevos de *Panstrongylus herreri* en el departamento de San Martin», *Rev. Méd. Per.*, Lima, **26**: 63-73, 1955.

36. Lumbreras, H., Arrarte, Juana, Guevara, Brígida, y Sipan, F. — «Observaciones preliminares sobre epidemiología de la enfermedad de Chagas en las provincias de Moyobamba y Rioja, del departamento de San Martin», *Rev. Méd. Per.*, Lima, **26**: 233-255, 1955.

37. Lumbreras, H., Estrada, A., Arrarte, Juana, y Tejada, A. — «Nota acerca de la presencia del *Panstrongylus herreri* y del *Triatoma carrioni* en el distrito de Querocotillo, provincia de Cutervo, del departamento de Cajamarca», *Rev. Méd. Per.*, Lima, **26**: 444-446, 1955.

38 Lumbreras, H., y Arrarte, Juana — «La infección natural y experimental del *Triatoma carrioni* por tripanosomas», *Arch. Per. Pat. Clín.*, Lima, **10**: 107-116, 1956.

39. Montesinos, J., Náquira, C., Ordoñez, J., Paredes, O., Rojas, V., Sevillano, G., Vargas, J., y Tejada, A. — «Epidemiología de la enfermedad de Chagas en el valle de Sihuas, provincia de Arequipa, departamento de Arequipa, 1956. Informe preliminar». Comunicación al Primer Congreso Americano de Estudiantes de Medicina. Lima, agosto de 1957.

40. Rubio D., Mafalda — «Estudio de una cepa de *Trypanosoma cruzi* aisla da de triatomídeos de una localidad del sur del Perú», *Rev. Biol.*, Santiago, **21**: 59-72, 1955.

41. Náquira, F., y Náquira, N. — «Contribución al estudio de la enfermedad de Chagas. Encuesta epidemiológica en el sur del Perú (provincia de Tarata, departamento de Tacna)», *Bol. Chileno Parasit.*, Santiago, **10**: 29-31, 1955.

42. Náquira, F., y Náquira, N. — «Contribución al estudio de la enfermedad de Chagas. II. Nuevas investigaciones epidemiológicas en el sur del Perú», *Bol. Chileno Parasit.*, Santiago, **12** (3): 46-50, 1957.

43. Ticse Alva, A. — *Observaciones experimentales sobre el ciclo biológico del* Triatoma infestans *(Klug, 1834)*. Tesis de Bachiller. Fac. Ciencias, Universidad Nacional Mayor de San Marcos, Lima, 1951.

44. Wygodzinsky, P. — «Sobre *Panstrongylus* del Perú, con la descripción de una nueva especie *(Triatoma, Reduviidae, Hemiptera)*», *An. Inst. Med. Reg.*, Tucuman, **2**: 197-208, 1948.

45. Zapatel Vasquez, J., y Acha Yanel, P. — *Indices triatomídeos y trypanotriatomídeos en el distrito de Jepelacio, provincia de Moyobamba, departamento de San Martin.* Folleto del Serv. Coop. Inter-Americano de S. P., Lima, 1955.

46. Zeledon, Rodrigo — «Trypanosomiasis *rangeli*», *Rev. Biol. Trop.*, Costa Rica, **2** (2): 231-268, 1954.

47. Varios autores — *Primera Reunión Panamericana Sobre Enfermedad de Chagas*, Tucuman, 1949.

# A DOENÇA DE CHAGAS EM PERNAMBUCO, BRASIL

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## INTRODUÇÃO

O Laboratório Regional do D. N. E. Ru., com sede no Recife, está realizando ampla investigação sobre a doença de Chagas nos cinco estados do Nordeste Oriental do País, compreendendo: *a*) inquérito entomológico e protozoológico para especificação dos triatomíneos existentes e determinação dos respectivos índices de infecção natural pelo *Trypanosoma cruzi* Chagas, 1909; *b*) investigação sorológica em amostra numerosa e representativa das várias zonas fisiográficas em que se divide cada estado; *c*) registro da casuística humana, comprovada pelas manifestações clínicas sabidamente decorrentes da infecção chagásica.

Em trabalhos anteriores publicámos o resultado do inquérito procedido no estado de Paraíba (Lucena & Costa, 1953, 1954, 1956 e 1957) nos moldes estabelecidos e aqui trazemos o resultado das pesquisas feitas em Pernambuco, expostas de maneira sucinta mercê do tempo exíguo.

O estado de Pernambuco mede 97 016 km². Duas terças partes do território são constituídas por solo semiárido; as chuvas são escassas e o clima é quente e seco — é o *sertão;* o restante compreende o *agreste,* com chuvas mais frequentes e clima tropical húmido, e o *litoral-mata,* com precipitações regulares e clima super-húmido. A população do estado, que é de 3 916 000 habitantes, adensa-se nas zonas mais próximas à costa, rarefazendo-se no sertão.

## INVESTIGAÇÃO ENTOMOPROTOZOOLÓGICA

Dos 90 municípios em que o estado se dividia por ocasião das investigações, 84 foram percorridos com a finalidade de descobrir os triatomíneos transmissores, capturá-los e deles examinar o conteúdo intestinal para descoberta de flagelados parasitas. Como resultado geral, tivemos 91,67 % dos municípios com triatomíneos, que em 70,24 % deles encontravam-se infectados pelo *Trypanosoma cruzi*.

QUADRO 1 — *Epidemiologia da doença de Chagas em Pernambuco — Municípios investigados*

| Zonas fisiográficas | Existentes em 1950 | Investigados | Com triatomíneos | Percentagem de municípios com triatomíneos | Com triatomíneos infectados | Percentagem de municípios com triatomíneos infectados |
|---|---|---|---|---|---|---|
| Litoral-mata . . . | 34 | 32 | 25 | 78,12 | 16 | 50,00 |
| Agreste . . . . . | 29 * | 27 * | 27 * | 100,00 | 20 * | 74,07 |
| Sertão . . . . . | 27 | 25 | 25 | 100,00 | 23 | 92,00 |
| *Total* . . . | *90* | *84* | *77* | *91,67* | *59* | *70,24* |

* Inclusive Alagoinha, que foi estudada como distrito de Pesqueira, sendo os dados incluídos neste.

Nesses 84 municípios foram pesquisadas 1 574 localidades, das quais em 705 havia triatomíneos (44,79 %) e em 282, isto é, 17,92 %, os hemípteros abrigavam o flagelado.

Dentre as 18 238 casas examinadas em todas as suas dependências, nos referidos municípios, em 1727 os insectos foram encontrados (9,47 %), sendo que infectados estavam os que se abrigavam em 442 delas (2,42 %).

Examinámos 15 579 triatomíneos de 15 666 capturados, pertencentes a sete espécies distintas (*P. megistus, T. brasiliensis, T. rubrofasciata, T. maculata, T. sordida, P. lutzi* e *T. melanocephala*), das quais cinco foram encontradas infectadas pelo *T. cruzi* na percentagem global de 13,14 %, conforme abaixo.

Das espécies transmissoras, o *P. megistus* espalha-se por quase todo o estado, enquanto o *T. brasiliensis* domina na zona do sertão, ambos sendo igualmente perigosos vectores. O *T. maculata,* embora

bastante difundido, é pouco abundante e o *T. rubrofasciata,* tão bom transmissor quanto as espécies autóctones ali onde se implantou, está limitado a 7 municípios do litoral-mata. O achado do *P. lutzi* infectado indica que as chamadas *espécies semidomésticas* têm importância na transmissão do protozoário.

QUADRO 2 — *Epidemiologia da doença de Chagas em Pernambuco — Localidades e casas investigadas*

| Regiões fisiográficas | Localidades | | | Casas | | |
|---|---|---|---|---|---|---|
| | Investigadas | Com triatomíneos | Percentagem | Examinadas | Com triatomíneos | Percentagem |
| Litoral-mata. . . . | 594 | 196 | 33,00 | 7 741 | 423 | 5,46 |
| Agreste. . . . . | 517 | 193 | 37,33 | 5 671 | 380 | 6,70 |
| Sertão . . . . . | 463 | 316 | 68,25 | 4 826 | 924 | 19,15 |
| *Total* . . . | *1 574* | *705* | *44,79* | *18 238* | *1 727* | *9,47* |

QUADRO 3 — *Índices de infecção dos triatomíneos pelo* T. cruzi *em Pernambuco*

| Espécies | Examinados | Infectados | Percentagem |
|---|---|---|---|
| 1. *P. megistus* . . . . . | 11 587 | 1 670 | 14,41 |
| 2. *T. brasiliensis* . . . . . | 2 932 | 311 | 10,61 |
| 3. *T. maculata* . . . . . | 513 | 40 | 7,80 |
| 4. *T. rubrofasciata*. . . . . | 542 | 26 | 4,80 |
| 5. *P. lutzi* . . . . . | 5 | 1 | 20,00 |
| *Total* . . . | *15 579* | *2 048* | *13,14* |

Vê-se, pois, que em todo o estado encontram-se triatomíneos vectores da doença de Chagas cujos índices de infecção pelo *T. cruzi* são particularmente severos, a traduzir uma endemia grave cuja repercussão humana já se vai conhecendo pela bibliografia clínica, que dia a dia mais se avoluma. Outra observação interessante resulta da análise das tabelas anexas, pelas quais se vê que os níveis endémicos vislumbrados através dos índices entomológicos e protozoológicos são distintos nas várias zonas fisiográficas em que o estado se divide, sobrelevando aqueles relativos ao sertão.

## SOROLOGIA DA DOENÇA DE CHAGAS EM PERNAMBUCO

A fim de comprovar a severidade da doença humana que os dados epidemiológicos já conhecidos sugeriam, procedemos a um inquérito sorológico amplo, colhendo sangue em amostras da população espalhada pelas diversas zonas fisiográficas do estado, de modo a ter um quadro sorológico que pudesse representar, na sua diversidade, as condições gerais de transmissão da doença ao homem.

Realizámos a reacção de Guerreiro-Machado pela técnica aconselhada pelo Prof. Júlio Moniz (1944), utilizando antígeno por ele próprio preparado, segundo o processo de Davis (1943). Considerámos positivas as reacções em que os tubos de prova apresentavam hemólise parcial, com nítido depósito de hematias de carneiro (++), até ausência completa de hemólise (+++,+).

Obtivemos 3 267 amostras de sangue de indivíduos de ambos os sexos e de todos os grupos etários representativos da população, cuja análise foi procedida em nosso laboratório, no Recife, de que damos abaixo os resultados, por municípios e no total.

Essa amostra, ainda pequena, traduz o nível médio de positividade de 14,88 %, índice seguro de infecção chagásica porque, como

QUADRO 4 — *Reacção de Guerreiro-Machado em Pernambuco*

| Número | Municípios | Soros examinados | Positivos mais de ++ | Percentagem de positividade |
|---|---|---|---|---|
| 1 | Limoeiro | 592 | 79 | 13,34 |
| 2 | Bom Jardim | 303 | 51 | 16,83 |
| 3 | Orobó | 321 | 55 | 17,13 |
| 4 | Surubim | 328 | 49 | 14,94 |
| 5 | Nazaré da Mata | 314 | 73 | 23,25 |
| 6 | Aliança | 299 | 51 | 17,06 |
| 7 | Catende | 325 | 40 | 12,31 |
| 8 | Palmares | 309 | 20 | 6,47 |
| 9 | Gameleira | 67 | 8 | 11,94 |
| 10 | Salgueiro | 206 | 29 | 14,08 |
| 11 | Ouricuri | 203 | 31 | 15,27 |
| | *Total* | *3 267* | *486* | *14,88* |

já acentuámos, representa apenas as reacções marcadamente positivas, com ++ a ++++.

O seu significado clínico já começa a ser comprovado, como veremos adiante, graças ao interesse que os médicos vêm tomando pelo assunto e às facilidades diagnósticas que são postas à sua disposição. Representa esse índice sorológico a evidência de uma infecção antiga, difundida por todo o estado, particularmente mais grave nas zonas mais áridas.

Passamos a estudar a repercussão mórbida da infecção chagásica no estado.

## CASUÍSTICA CLÍNICA

Quer sob a forma aguda, quer crónica, tem sido a doença de Chagas assinalada em Pernambuco desde 1940, quando descrevemos o primeiro caso em uma rapariga residente no interior do estado (Lucena, 1941). A partir de então a casuística vem sempre aumentando, sobretudo a relativa à forma crónica.

Quanto à forma aguda, a mais dramática na infância, pelo quadro infeccioso que se apresenta, a partir do primeiro caso que descrevemos em 1953 (Paiva, Lucena, Salazar & Marques, 1953), outros vêm se sucedendo, a demonstrar que a sua raridade é mais aparente que real. Os dois outros casos conhecidos, um de colega residente no interior do estado (Adejardo Silva, 1953), outro nosso, descoberto em trabalhos epidemiológicos de campo (Lucena & Marques, 1954), atestam a predominância da doença na zona rural, onde o médico ou o pesquisador devem se encontrar atentos se quiserem surpreender o doente nessa fase.

Sob a forma crónica, entretanto, já a entidade mórbida está hoje amplamente equiparada à nosologia regional, diagnosticada diàriamente nas clínicas privadas e nas instituições nosocomiais, graças à exploração semiológica cuidadosa e aos meios complementares esclarecedores, como o electrocardiograma, os raios X, a sorologia, o xenodiagnóstico.

Foi desse modo que os primeiros 23 casos foram publicados, revistos por Bezerra de Carvalho, em 1953, em relatório ao II Congresso Médico do Nordeste Brasileiro. Mais tarde, em 1955, Rui

Marques selecciona 80 casos clínicos de cardiopatias evidentes, megaesófago e megacólon, com que elabora magnífica tese de concurso. Recentemente (1957), esse mesmo autor e cols. trazem nova contribuição, descrevendo mais 40 casos novos de portadores de doença de Chagas com estudo electrocardiográfico acurado, devendo-se notar que «em todos os pacientes o diagnóstico foi estabelecido através da R. F. C.» (Marques, Tompson, Costa, Cordeiro & Saldanha, 1957). Por fim, Paulo Borba e cols. (1954) acrescentam 15 novos casos às suas observações anteriores, incluídas no estudo de Bezerra de Carvalho *(op. cit.)*. São, portanto, 158 casos clínicos suficientemente documentados que já constam da nossa bibliografia.

Graças à prática rotineira da reacção de Guerreiro-Machado em nosso laboratório a partir de 1955, temos facilitado a comprovação clínica de vários casos que se apresentam nos serviços do Hospital das Clínicas da Faculdade de Medicina de Recife, assim como nas clínicas privadas de colegas interessados. Com essa finalidade, já efectuámos 447 provas sorológicas, das quais 133 foram positivas, aumentando consideràvelmente a nossa casuística.

Damos, a seguir, a súmula das observações de 100 casos clínicos procedentes dos serviços já referidos, também resumidos no quadro 5. Infelizmente, 33 daquelas provas sorológicas deixam de ser aqui incluídas, à falta do competente registro clínico, extraviado na fonte.

QUADRO 5 — *Casos clínicos crónicos de doença de Chagas, comprovados sorològicamente, registados em nosocômios e clínicas privadas do Recife*

| Instituição | Forma cardiaca | Forma indeterminada | «Megas» Megaesófago, megacólon ou associações | Total |
|---|---|---|---|---|
| Instituto de Cardiologia | 38 | 9 | 4 | 49 |
| 2.ª cadeira de Clínica Médica | 22 | 2 | 1 | 25 |
| Clínica de Doenças Tropicais e Infectuosas | 3 | 2 | 2 | 9 |
| Clínica Neurológica | — | 2 | — | 2 |
| Clínica Propedêutica Médica | 3 | — | — | 3 |
| Clínicas privadas | 6 | — | 6 | 12 |
| *Total* | *72* | *15* | *13* | *100* |

Desse modo vê-se que, nesses últimos cinco anos, já é bastante expressivo o registro clínico da doença de Chagas em Pernambuco, decorrente de simples documentação hospitalar ou de clínicas privadas. Considerando-se a dificuldade antes existente para comprovação sorológica da entidade mórbida e não haver sido feito, até agora, amplo inquérito clínico na zona rural com triagem electrocardiográfica, que constitui mesmo a nossa rotina, a ser executada pròximamente, os 258 casos arrolados comprovam sobejamente a ocorrência da endemia.

De todo o exposto é lícito concluir que a doença de Chagas constitui problema de saúde pública grave também em Pernambuco e, provàvelmente, em todo o Nordeste Oriental do Brasil.

## SUMMARY

A complete survey on Chagas' disease was started in the State of Pernambuco, Brazil, to clarify the importance of this disease in the local nosology. In previous papers (in press) geografic distribution and ecological notes on the species of *Triatominae* captured were studied. Here are presented *a*) the indices of infection of triatome bugs collected and related to countries, localities and houses in which they had been found naturally infected with *Trypanosoma cruzi, b*) results of a serological survey, and c) cinical cases reported in hospitals and private clinics in Recife.

1. The following are the main records for the whole area surveyed:

COUNTIES

| | |
|---|---|
| In the whole State | 90 |
| Surveyed | 84 |
| With *Triatominae* | 77 |
| Percentage with *Triatominae* | 91.67 % |
| With *Triatominae* infected | 59 |
| Percentage with *Triatominae* infected | 70.24 % |

LOCALITIES

| | |
|---|---|
| Surveyed | 1,574 |
| With *Triatominae* | 705 |
| Percentage of positive | 44.75 % |
| With *Triatominae* infected | 282 |
| Percentage with *Triatominae* infected | 17.92 % |

HOUSES

| | |
|---|---|
| Examined | 18,238 |
| With *Triatominae* | 1,727 |
| Percentage of positive | 9.46 % |
| With *Triatominae* infected | 442 |
| Percentage with *Triatominae* infected | 2.42 % |

«TRIATOMINAE»

| | |
|---|---|
| Collected | 15,666 |
| Dissected | 15,579 |
| Percentage not examined | 0.56 % |
| Infected with *T. cruzi* | 2,048 |
| Percentage of positive | 13.14 % |

These epidemiological indices for the whole State have distinct levels if we consider the different zones in which the State is physiographically divided. As we had pointed out in a previous paper (in press), in the «sertão» zone «there are more favourable conditions to transmission of Chagas' disease», what is largely emphasized by the following figures:

| Physiographical zones | Percentage of counties with *Triatominae* | Percentage of counties with *Triatominae* infected | Percentage of localities with *Triatominae* infected | Percentage of houses with *Triatominae* infected | Percentage of *Triatominae* infected |
|---|---|---|---|---|---|
| Litoral-mata | 78.12 | 50.00 | 11.45 | 1.37 | 13.20 |
| Agreste | 100.00 | 74.07 | 9.86 | 1.04 | 9.55 |
| Sertão | 100.00 | 92.00 | 35.20 | 5.74 | 14.81 |
| *Total* | *91.67* | *70.24* | *17.92* | *2.42* | *13.14* |

These data agree with clinical reports registering human cases of cardiac involvement and megaesophagus prevalent in patients coming from that region. There is another sub-region, in northeastern part of the Stata («mata seca»), in which the percentage of *Triatominae* infected is of 28.21 %, from where human cases of Chagas' disease is enough frequent as well.

Dissection and examination of rectal contents of 15,579 triatome bugs collected gave the following percentage of infection: *P. megistus*, 14.41 %; *T. brasiliensis*, 10.61 %; *T. maculata*, 7.80 %; *T. rubrofasciata*, 4.80 %; *P. lutzi*, 20.00 %. *T. sordida* (8 specimens) and *T. melanocephala* (1 specimen) were negative.

2. In order to determine the human prevalence of Chagas' disease in the State, a serological survey was carried by obtaining blood samples of people living in the three main physiographical zones. Guerreiro-Machado complement fixation test was performed by using an antigen prepared according to Júlio Moniz, whose technic was followed to make the reactions.

A total of 3,267 sera were examined, from which 486 gave marked results. As indicative of Chagas' disease infection were considered those reactions with results varying from a partial hemolysis (++) to a complete absence of it (++++), what means, according to the above data, an index of infection of 14.88 % (see table 4 on the text).

3. Clinical cases of the disease have been reported since 1941, when we described the first subacute one. The first acute case was detected in 1953 and two others related thereafter.

Chronic cases represented by cardiac involvement, megaesophagus and megacholon have been reported in papers published, in a total of 158. Here are registered 100 cases more from the Clinic Hospital of the Faculty of Medicine of Recife and from private clinics, whose diagnose was established on clinical symptoms confirmed by serological test performed in our laboratory.

## BIBLIOGRAFIA


Bezerra de Carvalho, S. — «Moléstia de Chagas no Nordeste brasileiro», *An. II Cong. Méd. do Nord. Bras.*, pp. 41-58, 1953.

Borba, P., e outros — «Megaesófago, megacólon e doença de Chagas», *An. Soc. Med. Pernambuco*, **6** (1-2): 34-41, 1954.

Davis, D. J. — «An improved antigen for complement fixation in American trypanosomiasis», *Public Health Report*, **58** (20): 775-777, 1943.

Lucena, D. T. — «Primeiro caso de moléstia de Chagas em Pernambuco», *A Folha Médica*, número de 5 de Março de 1941.

Lucena, D. T., & Costa, L. — «Estudos preliminares sobre a doença de Chagas na Paraíba», *Rev. Bras. Malar. Doenç. Trop.*, **5** (1): 55-68, 1953.

Lucena, D. T., & Costa, L. — «Epidemiologia da doença de Chagas na Paraíba», *Rev. Bras. Malar. Doenç. Trop.*, **6** (2): 229-265, 1954.

Lucena, D. T., & Costa, L. — «Epidemiologia da doença de Chagas na Paraíba. Resultados parciais do inquérito sorológico», *Rev. Bras. Med.*, **13** (2): 90-92, 1956.

Lucena, D. T., & Costa, L.— «Inquérito sorológico sobre a doença de Chagas na Paraíba», *Rev. Bras. Med.*, **14** (5): 323-327, 1957.

Lucena, D. T., & Marques, R. J. — «Mais um caso agudo (3.°) de doença de Chagas em Pernambuco», *Rev. Bras. Med.*, **11** (12): 859-861, 1954.

Moniz, J., & Freitas, G. — «Contribuição para o diagnóstico da doença de Chagas pelas reacções de imunidade», *Mem. Inst. Osw. Cruz*, **41** (2): 303-333, 1944.

MARQUES, R. J. — *Alguns Aspectos da Doença de Chagas em Pernambuco*, Tese, Recife, 1955.

MARQUES, R. J., e outros — «Estudo electrocardiográfico da doença de Chagas em Pernambuco», *An. Fac. Med. Univ. Recife*, **17** (1): 75-79, 1957.

PAIVA, C., LUCENA, D. T., SALAZAR, R., & MARQUES, R. J. — «Primeiro caso agudo da doença de Chagas em Pernambuco», *Rev. Bras. Med.*, **10** (5): 417-419, 1953.

SILVA, A. — «Um caso agudo de doença de Chagas», *O Hospital*, **44** (5): 675-681, 1953.

## GENERAL DISCUSSION

Dr. P. C. C. Garnham (U.K.) — «Mal do engasgo» is supposed to have a Chagasian aetiology. What is the opinion of the speaker on this point?

Dr. P. C. C. Garnham (U.K.) — *To Dr. F. Pifano:* Is the question of the pathogenicity of *T. rangeli* to man settled?

Dr. Durval de Lucena (Brasil) — *Ao Dr. Garnham:* As áreas de incidência do «mal do engasgo» e da doença de Chagas são idênticas no Nordeste do Brasil.

Os numerosos casos de megaesófago e megacólon diagnosticados clìnicamente são sempre comprovados sorològicamente, de ordinário com reacções fortemente positivas.

A prova anátomo-patológica, aparentemente, já foi dada pelo Dr. Fritz Koeberle, que demonstrou a localização de leishmânias na musculatura esofagiana, conduzindo à destruição do plexo nervoso, que causa a descoordenação dos movimentos peristálticos.

As gastrites e enterites descritas por Porto (1955) no interior do Brasil, de natureza chagásica, merecem ser bem estudadas, sobretudo depois do trabalho de Lesser & Luckeman (1957) que provou o desenvolvimento de uma gastrite necrótica em camondongos experimentalmente infectados pelo *T. cruzi.*

Dr. Herman Lent (Brasil) — Desejo expressar meu desalento a respeito dos votos expressos nos congressos internacionais, embora considere imprescindível fixar bem a responsabilidade da Repartição Sanitária Panamericana.

A erradicação do *Aedes aegypti* é realizada pela Repartição em todos os países da América Latina, mas essa mesma erradicação não é feita no território dos Estados Unidos da América, onde a incidên-

cia desse mosquito é elevada. Esse exemplo serve para deixar patente o desalento acima expresso.

Dr. Félix Pifano (Venezuela) — No existe evidencia de patogenicidad del *Trypanosoma rangeli* en el material humano examinado por nosotros en Venezuela.

Dr. Eduardo del Ponte (Argentina) — Lo que ha señalado el Dr. Espinoza para la ciudad de Guayaquil tiene mucha importancia. Muestra el comienzo de una endemia chagásica por adaptación de dos elementos biológicos importantes: el vector y el reservorio.

El vector, *T. dimidiata,* fué señalado por Campos en 1932, quien, en esa época, anunció el peligro que se aproximaba.

El reservorio, *Didelphis paraguayensis,* «raposa» en Ecaudor, «gaúcha» en Brasil, se acerca a las casas y vive en ellas en forma semi-domiciliaria.

Ambos agentes del complejo epidémico de esta enfermedad son los responsables de dicha enfermedad. Ello significa la posibilidad de aparecer la dolencia en otras regións, con otros vectores y otros reservorios.

Dr. Alberto Cornejo Donayre (Perú) — A propósito del interesante trabajo de los Drs. Tejera y Pifano sobre la trypanosomiasis humana producida por el *T. rangeli,* creo que ha de ser oportuno informar sobre las investigaciones que hemos efectuado en el departamento de Madre de Dios, en el Perú, durante el mes de julio de 1957.

En 1919 el Dr. E. Escomel describió el primer caso de enfermedad de Chagas en el Perú, señalando que el enfermo procedía de la región del río Tahuamanu, en el departamento de Madre de Dios, y describió el trypanosoma por él encontrado con caracteres morfológicos que no correspondían al *T. cruzi,* es decir, longitud de 20 $\mu$ a 40 $\mu$ y blefaroblasto pequeno y apenas visible. Esto dió lugar a que Yorke considerase en marzo de 1920 que se trataba de una nueva especie y la denominó *T. escomeli.*

Ultimamente se ha sugerido que el *T. escomeli* sería en realidad el *T. rangeli* descrito por Tejera en julio de 1920 y que sería impor-

tante investigar la presencia de trypanosomiasis humana en el departamento de Madre de Dios.

En julio de 1957 efectuámos intensas investigaciones sobre triatóminos y enfermedad de Chagas en ese departamento de la selva peruana, que tiene más o menos 6.000 habitantes, con los siguientes resultados:

1. En todas las zonas pobladas de ese departamento hay condiciones epidemiológicas que favorecerían la diseminación de la enfermedad de Chagas, pues hay condiciones aparentes para la reproducción de triatóminos en las viviendas humanas, estas estan sobrepobladas y los habitantes viven en marcada promiscuidad con animales domésticos que en otros lugares sirven de reservorio al *T. cruzi.*

2. El examen de 591 personas de la población general, no seleccionada, no demostró la presencia de síntomas ni signos de la enfermedad de Chagas y el examen de frotises y gotas gruesas de sangre periférica de estas personas resultó negativo para *T. cruzi.*

3. No se encontró triatóminos en las zonas pobladas de la región del río Tahuamanu.

4. Se encontró *Rhodnius pallescens* en el interior de las viviendas humanas en la ciudad de Puerto Maldonado, capital del departamento de Madre de Dios y que está situada a enorme distancia del río Tahuamanu.

5. El *R. pallescens* encontrado estaba naturalmente infectado por un trypanosoma que, por sus características morfológicas y despues de inoculaciones experimentales en ratas jovenes, fué identificado como *T. cruzi.* No se encontró en la sangre de los animales inoculados ningún trypanosoma que pudiese tener semejanza con el *T. rangeli.*

6. *R. pallescens* naturalmente infectados y también los infectados experimentalmente eliminan con las heces formas de trypanosoma de tamaño normal y también formas de mayor tamaño, pero todas ellas con blefaroblasto grande y terminal.

7. Un regular número de xenodiagnósticos realizados, gracias a la colaboración del Dr. Neghme, resultó negativo en este mismo grupo de personas.

Dr. Luís Espinoza (Ecuador) — Agradezco mucho al Prof. Cecilio Romaña por su benévola felicitación por mi trabajo presentado. En una sesión anterior, en una exposición que hizo al Prof. Pifano

sobre el uso de la cortisona, sus observaciones coincidentes con las mias en cierta parte me alegraron mucho.

Dr. Luís Espinoza (Ecuador) — Sugiero a Su Señoria que esta Asamblea eleve la siguiente recomendación técnica a una comisión de expertos en enfermedad de Chagas de la Organización Mundial de la Salud:

Que se considere el valor y la interpretación que debe darse a los distintos procedimientos de investigación de la enfermedad actualmente conocida a fin de permitir una verdadera evaluación del problema de la enfermedad en una área endémica.

Dr. Luís Espinoza (Ecuador) — Respeto la opinión del Doctor Romaña, pero siento manifestarle que mi país el año pasado hizo un pedido formal de ayuda técnica y económica para organizar un programa de erradicación de la enfermedad de Chagas en Guayaquil, recibiendo una respuesta negativa.

Yo estimo, y esa es la opinión de nuestros países, que son los organismos mundiales de salud quienes, recogiendo los valiosos elementos de juicio que significan nuestras investigaciones, deben planear y organizar los programas de lucha contra las endemias en el mundo y someterlas a la consideración de los Gobiernos para coordinar la ayuda económica, ya que el aspecto técnico tiene que estar indudablemente en los organismos de salud.

Por eso es que insisto en que son los organismos de salud mundiales quienes deben llevar la iniciativa en todas las medidas contra las enfermedades en el mundo.

Dr. Cecilio Romaña (Argentina) — Como consecuencia de las conclusiones técnicas de la Sección Tripanosomiasis sobre la posibilidad de realizar programas de control y erradicación de la enfermedad de Chagas en los países donde la dolencia humana es endémica, los VI Congresos de Medicina Tropical y Paludismo recomiendan a los Gobiernos de América Latina que por intermedio de la O. S. P. inicien un programa internacional de lucha contra la endemia.

Dr. H. Lumbreras (Perú) — En relación al pedido formulado por el Dr. Cecilio Romaña, quisiera enmendarlo en el mismo sentido

en que lo hice el día 10 del actual al informar sobre la enfermedad de Chagas en el Perú.

Señalé en esa entonces que por ser la enfermedad de Chagas una endemia peligrosa en las Américas, convenía que los miembros de este VI Congreso de Medicina Tropical recomienden a los organismos internacionales de salud (O. M. S. y O. S. P.) para que no solo pongan su atención en este problema, sino que, a la brevedad posible, presten toda su ayuda técnica y económica para lograr su mejor conocimiento y, sobre esa base, estructurar los planes de una futura pero pronta erradicación.

Quiero aprovechar de esta circunstancia para señalar, asimismo, que el desconocimiento que se tenía de la enfermedad de Chagas en el Perú nos indujo, con el Dr. Pesce, a presentar el día 10 del actual una comunicación sucinta de todos los datos objetivos y numéricos consignados en la literatura publicada hasta fines de 1957. Las valiosas aportaciones dadas sobre esta misma materia por el Dr. Cornejo el día de hoy no son una omisión; él se ha referido a sus últimas investigaciones que hasta el momento actual mantenía inéditas.

Antes de terminar esta sesión quisiera referirme brevemente a unas experiencias que venimos realizando en el Instituto de Medicina Tropical de Hamburgo con los Drs. W. Florez y A. Escallón. Se trata de que hemos logrado reproducir experimentalmente el edema de los párpados o signal de Romaña por un mecanismo de sensibilización anafiláctico determinado por la saliva del *Rhodnius prolixus* no infectado por trypanosomas. Las manifestaciones clínicas objetivas, tal como se puede apreciar a través de los diapositivos que presento, son bastante típicas. Sin embargo, debo señalar que en dos experiencias que hemos realizado en sujetos diferentes el edema de los párpados duró solamente tres días y remitió expontáneamente, sin haberse apreciado infarto ganglionar preauricular. Como hemos reproducido otros tipos de reacción cutánea producidos por las mismas picaduras o por la inyección directa de saliva tanto de *R. prolixus* como del *T. infestans,* es conveniente que siga investigando sobre este asunto para decidir del valor que puede tener est tan conocido signal en el diagnóstico de las formas agudas de la enfermedad de Chagas.

SUBSECTION 2

## AMOEBIASIS

1ST SESSION: **The organism: «Entamoeba histolytica»**

*Monday, September 8th — 11:00 to 12:30 a.m. Room C*

The Chairman of the 1st session was Dr. Marcel Vaucel and the Rapporteurs were Dr. J. C. Basnuevo and Dr. R. Elsdon-Dew. The Secretary was Dr. L. T. de Almeida Franco.

The following papers were presented and discussed.

# THE PRESENT STATUS OF AMOEBIASIS

R. ELSDON-DEW, M.D., F.R.S., S.AF.

Director, Amoebiasis Research Unit [1].
Durban

Periodically in all fields of human endeavour there comes a time for a reassessment of our activities and for a reinterpretation of the information at our disposal. Too often have the teachings of some giant of the past been repeated from textbook to textbook until they are accepted without question. We must remember how the new seekers of the later renaissance were persecuted for daring to question the teachings of their masters.

So it is with amoebiasis. Over the years, many a misconception has become ingrained into our philosophy. The time has now come

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[1] The Amoebiasis Research Unit is sponsored by the following bodies:

The South African Council for Scientific and Industrial Research;
The Natal Provincial Administration;
The University of Natal;
The United States Public Health Service (Grant E-1592).

to sweep away the chaff and to set our «Augean Stables» in order. The present confusion in the minds of medical men about this disease must be dispelled. The picture must be clarified. This conference is an opportunity for the establishment of a sound foundation for our knowledge of the disease.

When we think back to the observations of the last century, we should feel ashamed that, with all our modern advantages, we have done so little since. One hundred and forty years ago, Annesley (2) noted the association between dysentery and abscess of the liver. It is 100 years since Docker (15) gave a factual report on the use of Ipecac following the lead of the South American Indians. Losch (3), in 1875, demonstrated the amoebae and was apparently able to transmit the disease to a dog. Passage of the amoeba to animals was also carried out in 1887 by Hlava (23), whose work was attributed to the mythical author O. Uplavici. The work of Kartulis (29), following that of Koch (30), suggested the aetiological part played by the amoeba in liver abscess.

Councilman and Lafleur (9), in their famous monograph from the Johns Hopkins Hospital in 1891, gave a comprehensive description of dysentery and of liver abscess. Their obervations have hardly been improved on to this day. They noted the occurrence of liver abscess without antecedent dysentery; they noted the necrotising action of the amoebae and the absence of purulent inflammation in the liver. Quincke and Roos (38) and, 10 years after them, Huber (27) were able to demonstrate the cystic forms of the amoeba. In distinguishing between harmless and pathogenic amoebae, Schaudinn (42), in 1903, coined the name *Entamoeba histolytica*. Though there was some criticism of the adoption of this name, it is now universally accepted. The next landmark in the story was the series of papers by Sir Leonard Rogers (40, 41) on the use of emetine, the active alkaloid of Ipecac, in the treatment of both dysentery and liver abscess.

The classical paper by Walker and Sellards (44) followed. These finally confirmed the distinction between *E. histolytica* and *E. coli*. There had been a series of papers describing new types of amoebae from all over the world. Dobell (12), in his monograph, lists over 30 names which he consider synonymous with *E. histolytica*. Of these terms, *E. minuta* Elmassian, 1909 (16), and *E. hartmanni*, v. Prowazek, 1912 (37), remain with us and their exact taxonomy is one

of the problems for this conference. Dobell's (12) *Amoebae Living in Man* has been the classical standard upon which the majority of modern workers have based their interpretation, but there has been fresh evidence since then.

Kuenen and Swellengrebel (31), in 1913, were the first to postulate a commensal phase in the life of *E. histolytica*. This phase they called the *minuta* stage, basing this name on the previous observations by Elmassian (16), who had thought he was dealing with a different species. In their infectivity experiments, Walker and Sellards (44) found that some of the cases they were able to infect showed no symptomatology, and apparently the amoebae were living free in the bowel and feeding *inter alia* on the bacteria. Dobell (12), in 1919, contested this, stating: «There is no evidence whatsoever that the *E. histolytica* depends for its existence on bacteria of any sort. It does not eat bacteria like the free-living amoebae and no concrete evidence has ever been brought forward to show that it lives in symbiosis, properly so-called, with any other organism ... *E. histolytica,* although a tissue parasite, does not usually cause dysentery or any other clinical symptoms in its host. As it destroys the tissues these regenerate. The parasite and host lives in a state of equilibrium.»

Though Dobell (13) changed his views, Craig and Faust (11), in their widely-read *Clinical Parasitology,* state: «*Endamoeba histolytica* is essentially a parasite that invades and lives within the tissues of its host and there is no conclusive evidence that it can live in the lumen of the bowel for any extended period of time without causing lesion ... Strictly speaking, a healthy carrier of *E. histolytica* does not exist, for in all carriers cytolysis and necrosis of at least the superficial layer of the mucosa are continuously occurring.» Craig (10) states: «The theory that *E. histolytica* can live indefinitely in the intestine is negatived by abundant evidence. The organism is essentially a tissue-parasite and the evidence is incontrovertible that it produces pathological lesions in the intestine, in other organs and tissues of the body, even in the absence of symptoms.» In his *Animal Agents and Vectors of Human Disease,* in 1955, Faust (21) states: «Infection with *E. histolytica* implies colonisation.» When such categorical statements are made by recognised authorities, it is not surprising that their conceptions gain wide-spread acceptance.

On the other hand, Brumpt (7), in Europe, postulated that there were two species of amoebae producing indistinguishable quadrinucleate cysts, one of which he called *E. dysenteriae* and the other *E. dispar,* the former being pathogenic and the latter never. This concept would help to explain the fact that in the temperate zones the presence of the *histolytica*-like amoeba is not associated with symptomatology. Additional evidence was brought by Reichenow (39), who pointed out that the enormous number of cysts discharged by symptomless carriers is far too great for these to be without pathology if the amoebae were indeed tissue-feeders. There has been the additional complication of the small amoeba of similar morphology which is regarded as a species *E. hartmanni* by Burrows (8) and others (22).

It is to Hoare that we owe some clarification of the whole issue. His series of papers (24, 25, 26) has done much to place our understanding of the epidemiology and clinical presentation of infection with these parasites on a proper basis. He states: «A critical analysis of the available data leaves no doubt that in symptomless carriers, as well as in latent periods of chronic infection, the amoebae live in the lumen of the gut and feed, among other faecal elements, on bacteria. Apart from their food habits, these trophozoites known as the *minuta* form can be distinguished from the haematophagous amoebae found in dysenteric patients by their smaller size. As they are the only amoebae capable of producing cysts, the *minuta* forms represent the essential phase of *E. histolytica* whereas the haematophagous amoebae have no lace in its normal life cycle.»

The views of Hoare (26), so succinctly put, were readily acceptable to a large proportion of workers on the subject, but they have not as yet reached the level of the practising physician whose approach is, it is to be regretted, still influenced by the conception that wherever there is *E. histolytica,* there is pathology.

There can be little doubt that this outlook has completely confused the relationship between *E. histolytica* and the disease. In many parts of the world, and it would be invidious to specify, the mere association of *E. histolytica* and some clinical conditions has been sufficient to imply the causal relationship of the parasite. Once the practising physican has become amoeba-conscious, it becomes facile for him to correlate the finding of the parasite with varied clinical

presentations by the patient. It may even reach the stage where «amoebiasis is a regular resort of the diagnostically destitute» (18).

This state of affairs has undoubtedly been stimulated in no small part by the inefficiency of the average laboratory diagnosis. In the diagnosis of *E. histolytica,* «the enthusiasm of the uninitiated is only to be matched by the caution of the expert» (17).

The analysis by Brooke (6) and his colleagues of the memorandum on the diagnosis of amoebiasis (48) served to highlight this very dangerous feature of modern practice. With regard to the identification of *E. histolytica* in faeces, the authors state: «From the lack of agreement relative to this subject, one wonders by what means the laboratory diagnosis has been accomplished during the past 50 years or more ...» All this adds to the confusion. McHardy (34) states: «Diagnostic inaccuracy, erroneous correlation of unrelated neurogenic symptoms to simple *E. histolytica* parasitisation, prognostic disparagement and fallacious depreciation of effective amoebicides in summation are productive of iatrogenic disease in relation to amoebiasis.» Adams (1) states: «It seems entirely unwarrantable, as is the practice of certain workers, more particularly in France and in the United States, to ascribe to an *E. histolytica* bowel infestation apparently limitless indeterminate symptoms remote from the abdomen ... One's credulity is stretched beyond the limit when anything from headache and visual, disturbance to sexual disorders and rheumatoid arthritis have been attributed to an otherwise symptomless *E. histolytica* infection solely on the grounds that specific treatment of the latter relieves these troubles.» Jones (28) believes that in neurotic patients the symptomatology of amoebiasis is often personality conditioned. It seems probable that in many areas «amoebophobia» is a worse disease than amoebiasis itself.

Here the responsibility must rest first with the physician and beyond him with us. The present position is that in many parts of the world everything from the lassitude of the tropics to iridocylitis is attributed to the amoeba. Perhaps the only thing for which the amoeba has not been blamed is pregnancy.

Thus, once again, it is up to us at this conference to establish the true relationship between *E. histolytica* and its manifestations and, by so doing, be able to prevent almost as much suffering as if we had discovered the complete cure for the disease.

If we accept the fact that in many parts of the world *E. histolytica* is seldom pathogenic, we have on the other hand to admit that in other parts of the world the amoeba does cause serious morbidity and mortality.

In our own part of the world, literally thousands of cases of acute amoebic dysentery present each year. There are myriads of actively motile, haematophagous amoebae, and the bowel is frequently affected from caecum to sigmoid. The mortality in the severe cases is high, commonly from diffuse peritonitis. Liver abscess is also common, and rupture into the lung and pericardium are not infrequent complications. Now is this due to the same amoeba which exists harmlessly in the bowels of 10 % of the population of Great Britain (24). Is the concept put forward by Brumpt (7) correct and the amoeba of the temperate zones is a different species, *E. dispar,* and that of the tropics, *E. dysenteriae,* or are these two amoebae merely strains of *E. histolytica?* If they are strains, are they interchangeable or not? If they are not interchangeable, perhaps we should regard them as different species, but if they are interchangeable, what is the trigger which changes the erstwhile commensal amoeba into an invasive form? There have been many suggestions.

Many authors (14, 45) have felt that the bacterial content of the bowel may be responsible. *In vitro* the amoeba refuses to grow in the absence of some symbiont, and it may be that in the bowel some bacterium may create conditions which induce the amoeba to invade. The enormous success of the antibacterial approach (3, 4) to therapy of bowel infestations indicates that the bacteria may play some part. The classical experiment of Westphal (45), though it implied but a single case, may well be a pointer to the mechanism involved.

The presence of other parasites in the bowel may also play some part in triggering invasion. Why is it that the ingestion of sewage, as has occurred in some of the epidemics (32), suddenly potentiated the otherwise harmless amoebae of a population to invade? Here too is another question for us to answer.

Does diet play any part in the genesis of the disease? The figures from Durban (17) suggest that a diet high in purified carbohydrate without supporting protein may well be a predisposing factor. Jane

Taylor (43) has shown the simpler the type of dietary carbohydrate, the higher is the incidence of the disease in infected rats. We (19) have shown that populations on a diet with adequate protein show a much lower incidence of the protozoa in general than do those on inadequate protein. It seems that the diet may so modify the bacterial flora of the bowel as to have a considerable effect on its fauna.

Why does the amoeba sometimes jump to the liver with or without previous bowel symptomatology? You will recall that some of the very early writers (2) were not sure which came first: the tropical liver or the dysentery. Amoebic abscesses not infrequently arise in the course of an amoebic dysentery, but almost as frequently they arise out of the blue with no antecedent history whatsoever. This is something of which we must know more. Is the liver in some way preconditioned to this invasion?

The serology and the immune response in amoebiasis is also a subject which needs clarification. There was always the hope that some such test might avoid the necessity for prolonged and meticulous examination of faecal specimens and yet the correlation between the finding of amoebae and positive serology is but slight. There have been many attempts at the production of antigens suitable for complement fixation or other reactions. Despite the early promise of the results shown by Craig (10) and others (5), it has been shown (20) that a large proportion of people harbouring invasive amoebae show no complement-fixing antibodies in their blood. Such failure has been attributed to strain differences in the amoebae, but may it not be that the patient just doesn't produce detectable antibodies? May it not be that we are dealing with some complex between amoeba and tissue and in part that the phenomenon is to be related to auto-immunisation and sensitisation. Tests other than complement fixation have been tried (35), but as yet none of these have achieved acceptance. That there is some lesson to be learned from the serology there is no doubt, but we must know a lot more of the mechanism before we can place any clinical reliance on random results.

The singular lack of response on the part of the host to invasion by the amoebae must prove something of an enigma to the histopathologists and we have obviously still got a lot to learn. Perhaps the most intriguing condition is that known as «amoebic hepatitis» implying a diffuse non-suppurative hepatitis of amoebic origin. We

do know that the liver not infrequently enlarges in the course of amoebic colitis, but is this a process specific to the amoeba or common to other forms of colitis? What is the relationship of this condition to the formation of liver abscess?

The laboratory study of the amoeba is also an intriguing problem. It has been mentioned that the amoeba will not grow without the presence of some other living tissue. Is the amoeba an anaerobe? What are the factors inducing encystment *in vitro?* With what does the bacteria supply the amoeba? What is the significance of the germ-free animals infected by Phillips (36)? What we have to do is to find the factor which the amoeba is apparently unable to elaborate for itself and this would be an immediate lead to specific therapy.

The very multiplicity of drugs used in the therapy of amoebiasis is evidence of our lack of knowledge in this direction. We are still using the drug suggested by the South American Indians, albeit in its purer form, emetine. This drug still holds pride of place in the treatment of extra-intestinal amoebiasis: though the advent of chloroquine has threatened its superiority in this field, it is still the drug of choice in liver abscess. Wilmot (47) has shown that the incidence of relapse under chloroquine is greater than that under emetine. Nevertheless, emetine is not always effective in the bowel and here it is that the wide-spectrum anti-bacterial attack has its place. There is nothing specific about any of the drugs for the combination of penicillin and a sulphonamide (3) is as effective as the tetracyclines, but it is apparent that these drugs act indirectly through the bacterial flora of the bowel, for they are ineffective in extra-intestinal amoebiasis, as has been shown by Wilmot (46). Even these drugs are not the answer for, despite the immediate cure rate and the apparent disappearance of the protozoa from the stool, there is still a significant relapse rate and there are of course the not unimportant side-effects. It may be that once the amoebae have got into the deeper tissues, they are no longer dependant on the bacteria for their unknown metabolite and thus they are no longer susceptible to the removal of their bacterial friends. We must obviously still attack the amoeba from the bowel and from the tissues and as yet we have no one drug which will do both.

## SUMMARY

Our conception of the disease amoebiasis is in a state of flux. A new appreciation of the taxonomy of the parasite is essential and we must learn to appreciate the significance of the commensal phase of *E. histolytica*. We must learn to establish better diagnostic methods and must be more sure of the association between the parasite and any pathology which we may attribute to it.

New methods of therapy are required, preferably some antimetabolite which will act both in and outside of the bowel. The whole condition bristles with problems and it is to be hoped that the papers and discussion at this Congress may throw some light upon them.

## BIBLIOGRAPHY

1. Adams, A. R. D. — *Brit. Med. J.*, **1**: 308, 1953.
2. Annesley, J. — *Researches into the Causes, Nature and Treatment of the More Prevalent Disease of India and of Warm Climates Generally*. Longman, Rees, Orme, Brown and Green, London, 1928.
3. Armstrong, T. G., Elsdon-Dew, R., and Marot, R. J. — *S. Afr. Med. J.*, **23**: 369, 1949.
4. Armstrong, T. G., Marot, J. T., and Elsdon-Dew, R. — *S. Afr. Med. J.*, **24**: 121, 1950.
5. Bozicevich, J., Hoyem, H. M., and Walston — *Publ. Hlth. Rep.* Washington. **61** (15): 529, 1946.
6. Brooke, M. M., Otto, G., Brady, F., Faust, E. C., Mackie, T. T., and Most, H. — *Am. J. Trop. Med. Hyg.*, **2**: 593, 1953.
7. Brumpt, E. — *Bull. Acad. Med.*, **94**: 943, 1925.
8. Burrows, R. B. — *Amer. J. Hyg.*, **65**: 172, 1957.
9. Councilman, W. T., and Lafleur, H. A. — *Johns Hopkins Hosp. Rep.*, **2**: 395, 1891.
10. Craig, C. F. — *The Etiology, Diagnosis and Treatment of Amoebiasis*. Williams and Wilkins Co. Baltimore, U.S.A., 1944.
11. Craig, C. F., and Faust, E. C. — *Clinical Parasitology*. Lea and Febiger, Philadelphia, 1943.
12. Dobell, C. — *The Amoebae Living in Man. A Zoological Monograph*. John Dale, London, 1919.
13. Dobell, C. — *Parasitol.*, **23**: 1, 1931.
14. Deschiens, R. — *Ann. Inst. Pasteur*, **61**: 5, 1938.
15. Docker, E. S. — *Lancet*, **2**: 113, 1858.
16. Elmassian, M. — *Zbl. Bakt., I. Abt. Orig.*, **52**: 335, 1909.
17. Elsdon-Dew, R. — *Am. J. Trop. Med.*, **29**: 337, 1949.
18. Elsdon-Dew, R. — *S. Afr. Med. J.*, **27**: 504, 1953.

19. Elsdon-Dew, R., and Horner, R. — *S. Afr. Med. J.*, **32**: 145, 1958.
20. Elsdon-Dew, R., and Maddison, S. E. — *J. Trop. Med. Hyg.*, **55**: 208, 1952.
21. Faust, E. C. — *Animal Agents and Vectors of Human Disease.* Lea and Febiger, Philadelphia, 1955.
22. Freedman, L., and Elsdon-Dew, R. — *Nature*, **181**: 433, 1958.
23. Hlava, J. — *Casopis Lékaruv Ceskych*, **26**: 70, 1887.
24. Hoare, C. A. — *Brit. Med. J.*, **2**: 238, 1950.
25. Hoare, C. A. — *Exp. Parasitol.*, **1**: 411, 1952.
26. Hoare, C. A., and Neal, R. A. — *Symposia of the Society for General Microbiology N.° 5, Mechanisms of Microbial Pathogenicity*, p. 230.
27. Huber, O. — *Deutsche Med. Wchnschr.*, **29**: 267, 1903.
28. Jones, C. A. — *J. Louisiana Med. Soc.*, **105**: 269, 1953.
29. Kartulis, S. — *Zbl. Bakt., I. Abt. Orig.*, **1**: 681, 1887.
30. Koch, R. — *Arb. a. d. k. Gsndhtsamte 30: Anlage VI*, p. 63, 1887.
31. Kuenen, W. A., and Swellengrebel, N. H. — *Zbl. Bakt., I. Abt. Orig.*, **71**: 378, 1913.
32. Le Maistre, C. A., Sappenfield, R., Culbertson, C., Carter, F. R. B., Offutt, A., Black, H., and Brooke, M. M. — *Am. J. Hyg.*, **64**: 30, 1956.
33. Lösch, F. — *Virchows Arch. Path. Anat.*, **65**: 196, 1875.
34. McHardy, G. — *Gastroenterology*, **25**: 616, 1953.
35 Moan, J. C. — *Am. J. Trop. Med. Hyg.*, **6**: 499, 1957.
36. Phillips, B. P., Wolfe, R. A., Rees, C. W., Gordon, H. A., Wright, W. H., and Reyniers, J. A. — *Am. J. Trop. Med. Hyg.*, **4**: 675, 1955.
37. Prowazek, S. Von — *Arch. Protistenk*, **26**: 241, 1912.
38. Quincke, H., and Roos, H. — *Berl. Klin. Wchnschr.*, **30**: 1089, 1893.
39. Reichenow, E. — *Arch. Schiffs-u. Tropenhyg.*, **41**: 257, 1937.
40. Rogers, Sir Leonard — *Phillipine J. Sci., B. Medical Sciences*, 219, 1910.
41. Rogers, Sir Leonard — *Brit. Med. J.*, **1**: 1424, 1912.
42. Schaudinn, F. — *Arb. a. d. k. Gsndhtsamte*, **19**: 547, 1903.
43. Taylor, D. J. — *J. Parasitol. Programme and Abstracts*, **36**: (6): Section 2, 21, N° 30, 1950.
44. Walker, E. L., and Sellards, A. W. — *Phillipine J. Sci., B. Tropical Medicine*, **8**: 253, 1913.
45. Westphal, A. — *Arch. f. Schiffs.-u. Trop.-Hyg.*, **41**: 262, 1937.
46. Wilmot, A. J., Armstrong, T. J., and Elsdon-Dew, R. — *Am. J. Trop. Med. Hyg.*, **1**: 429, 1952.
47. Wilmot, A. J., Powell, S. J., and Adams, E. B. — *Am. J. Trop. Med. Hyg.*, **7**: 197. 1958.
48. «Panel Discussion on Amoebiasis. Annual Meeting of Am. Soc. Trop. Med., Dec. 7, 1948», *Am. J. Trop. Med.*, **30**: 121, 1950.

# THE PATHOGENICITY OF *ENTAMOEBA HISTOLYTICA*

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*Entamoeba histolytica* comprises at least two races which differ in the size of their cysts. Cysts of the small race have a mean diameter of 7-8 $\mu$, while those of the large race have a mean diameter of 11-12 $\mu$; in addition they also have biological differences. The small race is more difficult to cultivate *in vitro* and is generally considered to be non-pathogenic. In consequence, some authors regard it as a separate species, *E. hartmanni*. The relationship of the large and small races is a separate problem and this paper deals solely with the variation of virulence of the large race.

One of the puzzling features accompanying infections with the large race of *E. histolytica* is the manner in which the relationship between host and protozoan may vary between commensalism and parasitism. As an illustration of this variability, the classical experiments of Walker and Sellards (1913) are often quoted. Since their work, a considerable amount of research has been carried out on this question, which has been ably summarized by several workers (Reichenow, 1937; Westphal, 1938*a, b;* Gnezdilov, 1941; Lavier, 1953; Anderson, Bostick & Johnson, 1953). In recent years a number of aspects have been re-investigated using new techniques. I propose to review this evidence and present a scheme of pathogenesis which might serve as a basis for further discussion and research [1].

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[1] In addition to the reviews noted above, my colleagues and I have recently presented fully documented reviews on various aspects of amoebiasis (Hoare & Neal, 1955; Neal & Vincent, 1955, 1956; Neal, 1956, 1957). In the present paper, therefore, only new or key references will be given.

## EVALUATION OF PATHOGENICITY

The virulence of *E. histolytica* is determined by inoculating animals and observing the degree of ulceration of the caecal mucosa. The difference in the degree of ulceration produced by invasive and avirulent strains is most marked if rats or rabbits are the experimental hosts and less easily observed if kittens are used (see also next section). Guinea pigs have also been employed, but there is no clear information at present on their susceptibility to strains of different virulence. At least 10 animals must be infected with a particular strain of *E. histolytica* to obtain a reliable estimate of its virulence.

Although this method is satisfactory, it would obviously be preferable to use a test which did not involve the presence of bacteria. It is necessary in conventional cultures to grow amoebae with bacteria, and the use of animals in the test introduces a further variable, since the caecal bacterial flora may vary from one animal to another. It is possible to use invasion of the liver (amoebae being injected directly into this organ) as an index of pathogenicity. This test possesses the theoretical advantage that the only bacteria present are those introduced with the amoebae. From the work of Yoshida (1952, 1953*a*, *b*), Sawada & Hara (1954*a*, *b*) and others, it seems that the animal of choice is the hamster. Bacteria do not appear to play a significant part in the production of hepatitis in these animals, and interchanging bacteria between invasive and non-invasive strains did not alter their virulence. Contrary to this result, which has been confirmed by several workers, it should be noted that Matsumoto (1956*a*) introduced new bacteria into a culture of virulent amoebae and found that the new association did not produce hepatitis. Ultimately, when the biochemical basis of virulence is understood, an *in vitro* test may become available.

## REVIEW OF FACTORS CONTRIBUTING TO THE PATHOGENICITY OF *E. HISTOLYTICA*

### Strain variation.

In earlier work the kitten was used for laboratory studies, but it is now known that this animal is unusually susceptible to the para-

site. Infection with *E. histolytica* nearly always results in ulceration of the large intestine and the host eventually dies. In spite of this hyper-susceptibility, two groups of workers claimed that differences in strain virulence could be detected. Brumpt claimed that amoebae from carriers were less virulent than those from acute cases; his views were supported by the work of Simic. Meleney and his colleagues confirmed that strain differences did exist and that the differences were stable over several years (Meleney, 1944). The precise significance of this difference in virulence was not clear, since other workers were unable to obtain marked differences; thus, Meleney & Frye (1937) reported that ulceration produced in kittens by carrier strains was as severe as that produced by virulent strains.

Recently this question has been re-examined using rats, guinea pigs and rabbits as experimental hosts. Experiments with these animals have revealed quite clearly that strains isolated from acute cases produced intense ulceration, while those from carriers either caused minimal ulceration or none at all (Schensnovich, 1955; Rogova, 1956; Hunninen & Boone, 1957; Neal, 1957). However, it was found that occasional strains isolated from patients without symptoms were highly invasive. Another important observation from these studies was that even avirulent strains occasionally produce caecal ulceration. This occurred irrespective of whether the animals were inoculated intracaecally with amoebae or orally with cysts of the same strain produced *in vitro*.

### Influence of bacteria.

When *E. histolytica* lives in the large intestine or in conventional cultures, the amoebae are closely associated with myriads of bacteria of diverse kinds. Much effort has therefore been expended on elucidating the role of bacterial flora in the invasion of the intestine wall. This work has recently been reviewed and two conclusions were drawn (Neal, 1956). First, if lesions are present in the intestine, it is reasonable to expect that bacteria will invade them. Secondly, interchanging bacterial floras of invasive and non-invasive strains did not alter the virulence of the amoebae (Matsumoto, 1956*b*). However, amoebae require growth factors from bacteria in order to grow *in vitro* and Phillips, Wolfe, Rees, Gordon, Wright & Reyniers (1955) showed

that an invasive strain would invade the caecal wall of guinea pigs in the presence of *Escherichia coli* or *Aerobacter aerogenes,* but would not infect the animals in the complete absence of bacteria. The presence of bacteria is essential for the survival and growth of amoebae in the intestines, but the part which they play in the production of intestinal lesions is not yet understood. Now, that axenic culture of *Entamoeba histolytica* has been achieved (Shaffer, Sienkiewicz & Washington, 1953; Reeves, Meleney & Frye, 1957; Baernstein, Rees & Bartgis, 1957), it should be possible to clarify this matter.

### Nutrition of the host.

It is conceivable that the state of nutrition of the host may contribute to the pathogenesis of *E. histolytica.* Thus Elsdon-Dew (1953) attributes the severe form of amoebic dysentery which occurs in Durban to the poor diet of the African. However, Carrera, Faust & Sadun (1952) did not observe any difference in degree of ulceration between guinea pigs fed upon a normal diet and those fed upon a protein-deficient diet. On the other hand, it is well known that dogs and cats are more susceptible to amoebic invasion of the intestinal wall when fed upon a high protein diet (cf. Poindexter, 1937).

Elsdon-Dew's hypothesis is supported by the finding that certain carbohydrates (sucrose, glucose, maltose, dextrin) promote caecal ulceration more than others (maize and rice starch, cellulose and laevulose) (Greenberg & Taylor, 1950). Deficiencies of ascorbic acid and nicotinic acid have also been studied (Sadun, Bradin & Faust, 1951; Larsh, 1952). In both cases the deficient animals were more susceptible to infection and showed an increased degree of ulceration. It has also been shown that two different diets (both presumably nutritionally complete) modified the degree of ulceration in guinea pigs and rats (Taylor, Greenberg & Josephson, 1952).

The precise manner in which the diets alter the susceptibility of the host is not known. Recently, Lynch (1957) made a histological study of the effect of an «ulceration-promoting diet» when fed to normal guinea pigs. He noted that there were distinct changes in the mucosa of the large intestine, including thinning and retention of secretions.

## Alteration of virulence by animal passage.

Further experimental evidence on the nature of virulence of *Entamoeba histolytica* has been gained by re-activating attenuated strains by passage *in vivo*. When invasive strains are cultured *in vitro*, they tend to lose their virulence, but repeated passage by intestinal infection of dogs and cats, or by liver infection in hamsters, restores the virulence to its original level. Employing the hamster liver technique, Neal and Vincent (1956) showed that the increase in virulence was not due to an increase in virulence of the bacterial flora, but presumably due to adaptation or selection of amoebae. However, it was also observed that avirulent strains could not be transformed into invasive strains by this method. On the basis of these results, I tentatively proposed that *E. histolytica* comprised two types differing only in virulence (Neal, 1957). However, this is not the only possible explanation; in fact, it seems to be contradicted by epidemiological evidence.

## Epidemiological evidence.

It is well known that *E. histolytica* occurs throughout the world, but in the temperate zone infections are asymptomatic. For instance, surveys of the incidence of intestinal protozoa in Britain (Yorke, Carter, Mackinnon, Matthews & Malin Smith, 1917; Morton, Neal & Sage, 1951) showed that about 5 % of the population were infected with *E. histolytica*. The infection is almost invariably of the carrier type, cases of dysentery being very rare. In the tropics, dysentery occurs frequently, but even in these areas infection with *E. histolytica* does not imply the presence of disease. Thus, Miller (1952) considered that the number of cases of amebic dysentery occurring in Calcutta was small in proportion to symptomless cases and Poindexter (1937) observed that dysentery due to *E. histolytica* was infrequent in Puerto Rico. Bearup and Lawrence (1950) observed that dysentery was rare in New Guinea though the incidence of intestinal amoebic infection was more than 14 %.

If there were at least two races or varieties of *E. histolytica* differing only in virulence, the geographical distribution of amoebic dysentery would suggest that the virulent race was confined to the tropics. However, since during 1914-18 and 1939-45 there were world-wide movements of troops, the virulent race should have been introduced into many new localities. In fact, Wenyon (1916) predicted the appearance of amoebic dysentery in the general population of Britain; these fears proved groundless and dysentery due to *E. histolytica* is still rare.

## CHANGE OF VIRULENCE

Although there is some experimental evidence against the view that avirulent strains can be transformed into invasive strains, I think that the epidemiological data outweighs the laboratory data so far available. It must be accepted that the virulence of *E. histolytica* can be modified, and if it is also accepted that the bacterial component plays little actual part in the invasion of the tissues by the amoebae, this means that virulent amoebae arise by some kind of adaptation from avirulent amoebae.

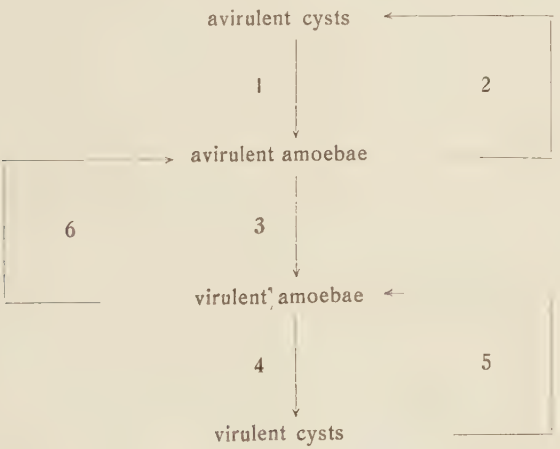


Fig. 1 — Hypothetical transformation of virulence of *Entamoeba histolytica*

Following this speculation still further, I have illustrated in the accompanying figure the modification of virulence which may occur. It represents a summary of experimental and epidemiological data.

Stages 1 and 2 represent the «normal» avirulent forms of *E. histolytica* which are associated with the carrier state in man. Strains of this type produce minimal or no ulceration in man. It is then postulated that, under certain (unknown) conditions, the amoebae become invasive (stage 3). Although we were unable to raise the virulence of non-invasive strains by liver passage, other methods may prove more successful. This possibility is supported by the fact that carrier strains will produce ulceration in some animals (see also above). If the clinical symptoms are mild, or if the patient is recovering from an acute attack, cysts may be passed in the stools. If these cysts, or amoebae grown from them *in vitro*, are inoculated into animals, ulceration is produced (stages 4 and 5). This may explain the occurrence of ulceration in animals by cysts passed by a patient who does not give a history of dysentery or diarrhoea. Treatment of patients suffering from dysentery often leads to loss of symptoms although *E. histolytica* is still present, which suggests that invasive characters have been lost (stage 6). This assumption is upheld by two facts: that virulent strains become attenuated by prolonged *in vitro* cultivation and that amoebic dysentery is rare in temperate climates (see also above). Experimental evidence of the loss of virulence (if it occurs) should be easily obtained by isolating cultures *in vitro* from a patient at intervals during clinical recovery from amoebic dysentery, the cultures being tested in animals for virulence.

A great deal of work is necessary before any theory on the nature of virulence, such as the one proposed here, can be accepted. In particular, the nature of the changes which occur when amoebae cease to be harmless lumen dwellers and invade the mucosa is unknown. The main difficulty is that it must account for the restriction of dysentery to tropic and subtropic regions and for the varying susceptibility of different experimental hosts. Westphal (1938*b*) postulated that bacterial injury to the epithelial cells is necessary before invasion can take place, but, if this is true, such bacteria must be confined to the geographical regions where dysentery occurs. Moreover, it has been shown experimentally that bacteria from invasive strains do not increase the virulence of avirulent strains. From these facts it seems unlikely that microscopic injury is the explanation. It is feasible, however, that a more subtle change, perhaps similar to that caused by the «ulceration-promoting diets» in guinea pigs (Lynch, 1957), or

variations of the cell membrane permeability or molecular structure, may contribute to or initiate the induction of invasiveness in *E. histolytica*.

Invasiveness is stable *in vitro* for many months, hence it must be transmitted to daughter amoebae after cell division. It should be remembered, however, that these amoebae reproduce solely by binary fission and it is not known whether encystation would modify the transmission of this character. This is obviously important since the cyst is the stage of the life history responsible for infecting new hosts.

## SUMMARY

1. The estimation of virulence is discussed and attention is directed to the possible use of experimental hepatitis as a test of invasiveness.

2. Experimental and epidemiological evidence is briefly reviewed and it is suggested that invasive amoebae may arise by adaptation from the normal avirulent amoebae.

## BIBLIOGRAPHY

ANDERSON, H. H., BOSTICK, W. L., & JOHNSTONE, H. G. — *Amebiasis*. Springfield, Illinois. C. C. Thomas, 1953.

BAERNSTEIN, H. D., REES, C. W., & BARTGIS, I. L. — «The rate of reproduction of *Entamoeba histolytica* in microcultures from inocula of single trophozoites in cell-free medium prepared from embryos of the chick», *J. Parasit.*, **43**: 143, 1957.

BEARUP, A. J., & LAWRENCE, J. J. — «A parasitological survey of five New Guinea villages», *Med. J. Australia*, **1**: 724, 1950.

CARRERA, G. M., SADUN, E. H., & FAUST, E. C. — «The effect of protein deficient diets on the susceptibility of guinea pigs to infection with *Entamoeba histolytica*», *Amer. J. trop. Med. Hyg.*, **1**: 966, 1952.

ELSDON-DEW, R. — «The pathogenicity of *Entamoeba histolytica*», *S. Afr. med. J.*, **27**: 504, 1953.

GNEZDILOV, V. G. — [«On the commensal and parasitic forms of the intestinal protozoa in connection with the question on their pathogenicity»], *Trav. Acad. Milit. Méd. Armée Rouge, U.R.S.S. Moscow*, **25**: 370, 1941. In Russian with English summary, abstract in *Trop. Dis. Bull.*, **40**: 911, 1943.

GREENBERG, J., & TAYLOR, D. J. — «The effect of dietary carbohydrate on *Endamoeba histolytica* infections in the rat», *J. Parasit.*, **36**: suppl., p. 21 [abstract], 1950.

HOARE, C. A., & NEAL, R. A. — «Host-parasite relations and pathogenesis in infections with *Entamoeba histolytica*», *Mechanisms of Microbial Pathogenicity, Symp. Soc. gen. Microbiol.*, **5**: 230, 1955.

HUNNINEN, A. V., & BOONE, H. A. — «Studies on the pathogenicity of various strains of *Entamoeba histolytica* in the rabbit», *Amer. J. trop. Med. Hyg.*, **6**: 32, 1957.

LARSH, J. E. — «The effect of a blacktongue-producing diet on experimental amoebiasis in dogs», *Amer. J. trop. Med. Hyg.*, **1**: 970, 1952.

LAVIER, G. — «Sur la pathogénicité de l'amibe dysentérique», *Bull. Acad. Nat. Méd. Paris*, **137**: 45, 1953.

LYNCH, J. E. — «Histological observations on the influence of a special diet used in experimental amoebiasis in guinea pigs», *Amer. J. trop. Med. Hyg.*, **6**: 813, 1957.

MATSUMOTO, H. — «Experimental amoebic infection of the liver in hamsters, with special reference to the influence of the alternation of associated bacterial flora upon the production of amoebic hepatitis», *Nisshin Igaku*, **43**: 292, 1956*a*. In Japanese with English summary.

MATSUMOTO, H. — «Experimental infection of *Entamoeba histolytica* in guinea pigs, with special reference to the effect of the alternation of associated bacterial flora upon the pathogenicity of the amoeba», *Nisshin Igaku*, **43**: 28, 1956*b*. In Japanese with English summary.

MELENEY, H. E. — «The relationship of clinical amoebiasis to various strains and the growth requirements of *Endamoeba histolytica*», *Puerto Rico J. publ. Hlth. trop. Med.*, **20**: 59, 1944.

MELENEY, H. E., & FRYE, W. W. — «The pathogenicity of four strains of *Endamoeba histolytica* from Chicago», *Amer. J. dig. Dis.*, **4**: 37, 1937.

MILLER, M. J. — «The experimental infection of *Macaca mulatta* with human strains of *Entamoeba histolytica*», *Amer. J. trop. Med. Hyg.*, **1**: 417, 1952.

MORTON, T. C., NEAL, R. A., & SAGE, M. — «Indigenous amoebiasis in Britain», *Lancet* (i), p. 766, 1951.

NEAL, R. A. — «Strain variation in *Entamoeba histolytica*. III. The influence of the bacterial flora on virulence to rats», *Parasitology*, **46**: 183, 1956.

NEAL, R. A. — «Virulence in *Entamoeba histolytica*», *Trans. R. Soc. trop. Med. Hyg.*, **51**: 313, 1957.

NEAL, R. A., & VINCENT, P. — «Strain variation in *Entamoeba histolytica*. I. Correlation of invasiveness in rats with clinical history and treatment of the experimental infections», *Parasitology*, **45**: 152, 1955.

NEAL, R. A., & VINCENT, P. — «Strain variation in *Entamoeba histolytica*. II. The effect of serial liver passage on the virulence», *Parasitology*, **46**: 173, 1956.

PHILLIPS, B. P., WOLFE, P. A., REES, C. W., GORDON, H. A., WRIGHT, W. H., & REYNIERS, J. A. — «Studies on the ameba-bacteria relationship in amebiasis», *Amer. J. trop. Med. Hyg.*, **4**: 675, 1955.

POINDEXTER, H. A. — «Some observations on the effects of environmental changes on the biology of *Endamoeba histolytica*», *Puerto Rico J. publ. Hlth. trop. Med.*, **12**: 314, 1937.

REEVES, R. E., MELENEY, H. E., & FRYE, W. W. — «Bacteria free cultures of *Entamoeba histolytica* with chick-embryo tissue juice», *Z. Tropenmed. u. Parasit.*, **8**: 213, 1957.

REICHENOW, E. — «Die Biologie der *Entamoeba histolytica* als Grundlage für die Pathogenese», *Arch Schiff.-u. Tropenhyg.*, **41**: 25, 1937.

ROGOVA, L. I. — [«Pathogenicity of strains of dysentery amoeba recovered from healthy carriers»], *Med. Parasit. Dis. Moscow*, **25**: 330, 1956. In Russian; abstract in *Trop. Dis. Bull.*, **54**: 572, 1957.

SADUN, E. H., BRADIN, JR., J. L., & FAUST, E. C. — «The effect of ascorbic acid deficiency on resistance of guinea pigs to infection with *Endamoeba histolytica* of human origin», *Amer. J. trop. Med.*, **31**: 426, 1951.

SAWADA, T., & HARA, K. — «Studies on the production of amebic liver abscess I. Experimental production of liver abscess in cats», *Gunma J. med. Sci.*, **3**: 169, 1954*a*.

SAWADA, T., & HARA, K. — «Studies on the production of amebic liver abscess. II. Experimental production of liver abscess in rabbits and dogs», *Gunma J. med. Sci.*, **3**: 181, 1954*b*.

SCHENSNOVICH, V. B. — [«Pathogenicity of strains of *Entamoeba histolytica* recovered from healthy carriers»], *Med. Parasit. Dis. Moscow*, **4**: 317, 1955. In Russian, *Trop. dis. Bull.*, **53**: 444, 1956.

SHAFFER, J. G., SIENKIEWICZ, H. S., WASHINGTON, J. E. — «The propagation of *Endamoeba histolytica* in tissue-bearing culture without accompanying bacteria or other microorganisms», *Amer. J. Hyg.*, **57**: 366, 1953.

TAYLOR, D. J., GREENBERG, J., & JOSEPHSON, E. S. — «The effect of two different diets on experimental amebiasis in the guinea pig and in the rat», *Amer. J. trop. Med. Hyg.*, **1**: 559, 1952.

WENYON, C. M. — «The protozoological findings in five hundred and fifty-six cases of intestinal disorder from the Eastern Mediterranean War area», *J. Roy. Army med. Corps*, **26**: 445, 1916.

WESTPHAL, A. — «Die Pathogenese der Amöbenruhr bei Mensch und Tier. I. Das Wesen der pathogenetischen Wirksamkeit der Ruhramöbe», *Arch. Schiffs.-u. Tropenhyg.*, **42**: 343, 1938*a*.

WESTPHAL, A. — «Die Pathogenese der Amöbenruhr bei Mensch und Tier. II. Die Pathogenese der Amöbenruhr beim Menschen», *Arch. Schiffs.-u. Tropenhyg.*, **42**: 441, 1938*b*.

YORKE, W., CARTER, H. F., MACKINNON, D. L., MATTHEWS, J. R., & SMITH, A. M. — «Persons who have never been out of Great Britain as carriers of *Entamoeba histolytica*», *Ann. trop. Med. Parasit.*, **11**: 87, 1917.

YOSHIMURA, B. — «Experimental studies on the amoebic liver abscess. I. Experimental amoebic liver abscess in rats», *Keio J. Med.*, **1**: 313, 1952.

YOSHIMURA, B. — «Experimental studies on the amoebic liver abscess. II. Influences of associated bacteria and the decrease of host-resistance upon the production of the abscess», *Keio J. Med.*, **2**: 39, 1953*a*.

YOSHIMURA, B. — «Experimental studies on the amoeba liver abscess. III. Effects of associated bacterial flora originating from the same and different host species upon the production of experimental amoebic liver abscess», *Keio J. Med.*, **2**: 109, 1953*b*.

# DIFFERENTIATION OF *ENTAMOEBA HARTMANNI* FROM THE DWARF STRAIN OF *ENTAMOEBA HISTOLYTICA*

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In a previous study (Burrows, 1957) it was shown tha there were various morphological features which differentiated *Entamoeba hartmanni* from *E. histolytica*. These features, together with the physiological differences noted by other investigators, were considered sufficient evidence that *E. hartmanni* could not be the so-called «small race o *E. histolytica*». In a later paper Freedman and Elsdon-Dew (1958) showed that in combined culture the two species remained distinguishable and were, therefore, genetically different.

In the earlier study it was shown that *E. histolytica* occasionally occurred in forms which were under 10 $\mu$ in diameter and this form was designated as «dwarf *E. histolytica*» to distinguish it from the larger forms, rather than retaining the term «small race», which actually included the dwarf forms as well as *E. hartmanni*. The present study is a morphological comparison between the trophozoites and cysts of *E. hartmanni* and the dwarf *E. histolytica*, bringing out those features which should make it possible for others to distinguish the two species. Only material from iron-hematoxylin stained fecal specimens was used, as this constitutes the most reliable means of differentiating the species.

## PROCEDURE

Preliminary sketches were made of over 50 trophozoites of each species and these were paired according to their approximate sizes and shapes. In order to get this number, it was necessary to examine hundreds of amebae and to select the larger *E. hartmanni* and the smaller dwarf *E. histolytica*. From this number, 10 pairs of typical amebae were selected for Fig. 1.

As cysts varied more in size than in shape, preliminary sketches were made of 25 cysts of each species, selecting the larger cysts of *E. hartmanni* and the smaller cysts of dwarf *E. histolytica*. From this number, 10 pairs of typical cysts were selected for Fig. 3.

Hundreds of trophozoites, cysts and their nuclei were measured. At the same time detailed observations were made of nuclei and sketches were made of a number. Some of the sketches were selected for Fig. 2.

## OBSERVATIONS ON TROPHOZOITES

*Size.* — Rounded trophozoites of *Entamoeba hartmanni* range from 3.0 $\mu$ to 10.5 $\mu$ in diameter, with the majority being under 7.0 $\mu$. Those of the dwarf *E. histolytica* range from 6.5 $\mu$ to over 11.0 $\mu$, merging in size with those of the large strain.

*Nucleus of «E. hartmanni».* — The nuclei range in diameter from 1.5 $\mu$ to 3.2 $\mu$, with the majority being between 2.0 $\mu$ and 2.5 $\mu$. The karyosome may be large or small and centric or eccentric. The peripheral chromatin consists of a few discrete bodies, which are not uniformly distributed on the nuclear membrane. In general, there is one prominent mass of chromatin and several smaller ones (Fig. 1 A, C, F, etc.). The largest chromatin mass may be in the form of a crescent (Fig. 1 C, G). In other nuclei the chromatin material may consist of fairly uniform bodies, but with wide gaps between them (Fig. 1 J). One peculiarity of the *E. hartmanni* nucleus is the position of the prominent peripheral chromatin mass. In some nuclei (Fig. 1 C, E) it appears to be inside the nuclear membrane; in others (Fig. 1 B, I) it appears to lie on both sides of the nuclear

membrane; and in some (Fig. 1 H) it appears to be on the outside of the membrane. Whether the chromatin material actually lies on the outside or on both sides of the membrane or whether the nucleo-

Fig. 1 — Comparative morphology of trophozoites of *Entamoeba hartmanni* and of dwarf *E. histolytica*. × 2500. A-J, *E. hartmanni;* a-j, *E. histolytica*. Details are given in the text

plasm forces the thick mass of chromatin and the nuclear membrane outward at this point has not been determined.

*Nucleus of the dwarf «E. histolytica».* — The nuclei range in diameter from 2.8 $\mu$ to 3.8 $\mu$, with the majority being between 3.0 $\mu$ and 3.5 $\mu$, or about 1.0 $\mu$ greater in diameter than the nucleus of *E. hartmanni*. The karyosome may be large or small and centric or eccentric. The peripheral chromatin generally is rather uniformly distributed around the nuclear membrane, being closely packed in some (Fig. 1 b, e) and less compact in others (Fig. 1 h, j). In many nuclei a portion of the chromatin material may be consolidated in a somewhat crescent-shaped mass (Fig. 1 d, h), but the consolidation is less prominent than in *E. hartmanni* and the remainder of the nuclear membrane has many, rather than few, chromatin granules. Some nuclei show several large granules among the smaller, uniformly distributed granules (Fig. 1 c, h).

*Peripheral chromatin of «E. hartmanni», «E. histolytica» and «E. coli».* — A comparison of the peripheral chromatin in these three species is shown somewhat diagrammatically in Fig. 2. In each pair of figures the left is an optical section through the center of the nucleus and the right is of the dorso-lateral surface of the nucleus. In *Entamoeba histolytica* (Fig. 2 A) the chromatin granules are uniformly distributed, both in the transverse section and in the lateral view. In *E. coli* (Fig. 2 B, C) the chromatin in transverse section shows masses which are irregular in shape and size. In lateral view the masses are seen to vary in size, shape and distribution. In the transverse section of *E. hartmanni* (Fig. 2 D-I) the chromatin consists of masses which vary in size and shape, but which are fewer in number than those of *E. coli*. In lateral view the large chromatin mass or masses extend upward or downward along the membrane (Fig. 2 E-G) and the two masses may meet and fuse (Fig. 2 D). These masses of chromatin resemble those of *E. coli*, but are generally larger in proportion to nuclear size. From these figures it is evident that the *E. hartmanni* trophozoite nucleus resembles that of *E. coli* much more closely than it does that of *E. histolytica*, as pointed out in a previous paper (Burrows, 1957).

*Cytoplasm.* — No differences were noted either in the character of the cytoplasm or in the ingested food. Both *E. hartmanni* and the

dwarf *E. histolytica* feed primarily on bacteria, but occasionally amebae of each species showed ingested yeast cells.

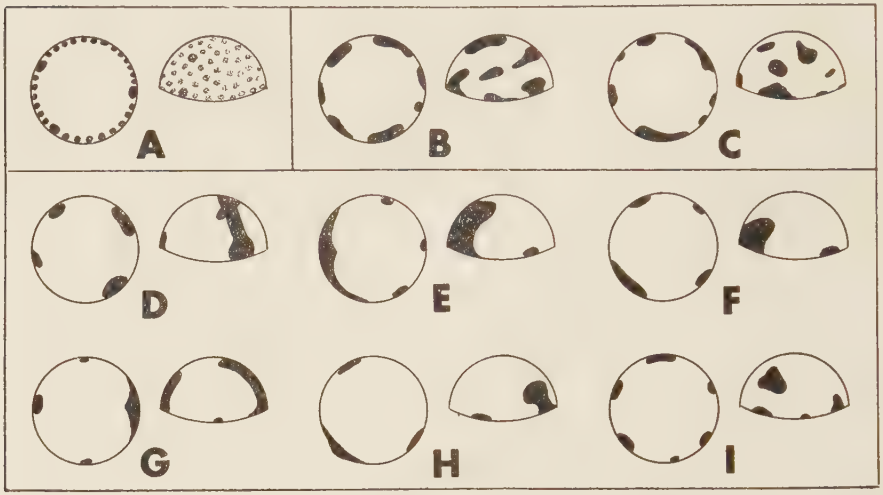


Fig. 2 — Distribution of peripheral chromatin in *Entamoeba histolytica*, *E. coli* and *E. hartmanni*. Semidiagrammatic and not to scale. A., *E. histolytica;* B, C, *E. coli;* D-I, *E. hartmanni*

## OBSERVATIONS ON CYSTS

*Size.* — Cysts of *E. hartmanni* range in diameter from 3.8 $\mu$ to 8.0 $\mu$, with the majority being less than 7.0 $\mu$. Those of the dwarf *E. histolytica* range from 5.5 $\mu$ to over 10.0 $\mu$, with the majority over 7.0 $\mu$. In size they merge into the range of the large strains.

*Uninucleate cysts.* — The nuclei of *E. hartmanni* range from 1.8 $\mu$ to 3.0 $\mu$ in diameter, with the majority being between 2.4 $\mu$ and 2.8 $\mu$. In general, the nucleus is from 25 % to 40 % of the cyst diameter. The nuclei of the dwarf *E. histolytica* are from 3.2 $\mu$ to 4.4 $\mu$ in diameter, with the majority averaging about 3.6 $\mu$ to 4.0 $\mu$. Most of these nuclei are from 45 % to 60 % of the cyst diameter. The nuclei of the uninucleate cysts of the dwarf form are somewhat similar in size to those of the large strain, but make up a larger percentage of cyst diameter due to the smaller size of the cyst. There

appear to be no distinctive differences in the distribution of the chromatin in the two species (Fig. 3). Frequently dwarf *E. histolytica* show the presence of large glycogen vacuoles (Fig. 3 a, d, g), which

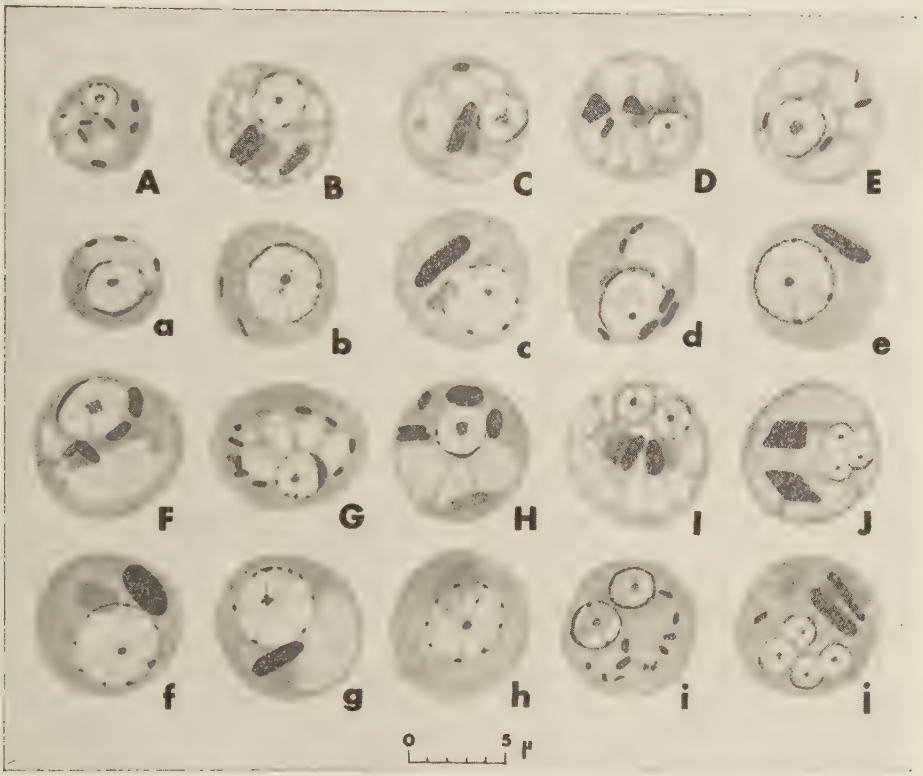


Fig. 3 — Comparative morphology of cysts of *Entamoeba hartmanni* and of dwarf *E. histolytica*. × 2500. A-J, *E. hartmanni*; a-j, *E. histolytica*. Details are given in the text

may occupy as much or more of the cyst than does the nucleus. Cysts of *E. hartmanni* rarely show glycogen vacuoles, but the cytoplasm is vacuolated much as in *Entamoeba polecki* cysts (Fig. 3). In some cysts (Fig. 3 A) only a few vacuoles may be present, although the majority of cysts show most of the cytoplasm to be filled with vacuoles (Fig. 3 B-H). These vacuoles do not stain deeply with

iodine as do the glycogen vacuoles of *E. histolytica*. There is considerable variation in chromatoidal bodies in both species and the two species cannot be differentiated on the basis of these structures.

*Bi- and tetranucleate cysts.* — In binucleate cysts of *E. hartmanni* the nuclei range from 1.3 $\mu$ to 2.0 $\mu$ in diameter and in the tetranucleate cysts they are from 0.7 $\mu$ to 1.7 $\mu$ in diameter. Those of dwarf *E. histolytica* are slightly larger, being from 2.0 $\mu$ to 2.8 $\mu$ in the binucleate cysts and from 1.4 $\mu$ to 2.2 $\mu$ in the tetranucleate cysts. In *E. hartmanni* the nuclear chromatin distribution closely resembles that found in the uninucleate cysts. However, in the binucleate cyst of the dwarf *E. histolytica* the distribution of the peripheral chromatin is generally like that of the trophozoite rather than of the uninucleate cyst (Fig. 3 i). Although glycogen vacuoles are sometimes found in binucleate cysts of dwarf *E. histolytica,* the remainder of the cytoplasm presents a uniform appearance as in the uninucleate cysts. In *E. hartmanni,* on the other hand, the cytoplasm of the bi- and tetranucleate cysts shows a distinct vacuolation, as in the uninucleate cysts.

## SIGNIFICANCE OF NUCLEAR SIZE

Inasmuch as there is a slight overlap in nuclear sizes in the two species, both in trophozoites and in cysts, there may be some question as to whether the differences in nuclear sizes are significant. From the numerous measurements which have been made, trophozoites of the two species were paired according to their diameters. For each *E. histolytica* the nuclear diameter was recorded in one column and in another column the nuclear diameter of *E. hartmanni* of that pair was recorded. For each column of nuclei the mean, the deviations from the mean and the square of the deviations were determined. From this data the *t* value for paired variates was computed. The same procedure was followed for the uni-, bi-, and tetranucleate cysts. The results are summarized in the table.

From this table it is obvious that the *t* values are quite significant and are as important as the other morphological variations found. Had a random selection been made, rather than pairing the amebae according to size, the *t* values would have been higher, for the larger

*E. hartmanni,* with somewhat larger nuclei, were necessary for pairing with dwarf *E. histolytica.*

t *values for nuclei of paired trophozoites and cysts of the two species*

| Stage | Number of pairs | Range and mean of nuclei (μ) | | t |
|---|---|---|---|---|
| | | *E. histolytica* | *E. hartmanni* | |
| Trophozoites . . . . . | 25 | 2.8-3.8<br>3.15 | 2.0-3.2<br>2.41 | 8.155 |
| Uninucleate cysts . . . . . | 25 | 3.2-4.4<br>3.80 | 2.0-3.0<br>2.60 | 13.451 |
| Binucleate cysts . . . . . | 15 | 2.2-2.9<br>2.59 | 1.5-2.0<br>1.71 | 11.331 |
| Tetranucleate cysts . . . . . | 15 | 1.6-1.8<br>1.68 | 1.1-1.4<br>1.25 | 12.490 |

## SUMMARY AND CONCLUSIONS

The major features which distinguish *Entamoeba hartmanni* from the dwarf *E. histolytica* may be summarized as follows:

1. The majority of *E. hartmanni* trophozoites are under 7.0 μ in diameter, whereas practically all of those of the dwarf *E. histolytica* are over 7.0 μ.

2. The *E. hartmanni* trophozoite possesses a smaller nucleus, which shows an *E. coli* type of peripheral chromatin distribution rather than a uniform distribution of small granules as in *E. histolytica.*

3. Uninucleate cysts of *E. hartmanni* possess nuclei which are 25 % to 40 % of the cyst diameter, whereas the nuclei of uninucleate dwarf *E. histolytica* are 45 % to 60 % of the cyst diameter. Nuclei of bi- and tetranucleate cysts of *E. hartmanni* are correspondingly smaller than those of dwarf *E. histolytica.*

4. Glycogen vacuoles are frequent in dwarf *E. histolytica,* but quite rare in *E. hartmanni.*

5. *E. hartmanni* cysts show an extensively vacuolated cytoplasm, much like that found in *E. polecki* cysts.

6. Although there is a slight overlap in the nuclear size ranges of *E. hartmanni* and dwarf *E. histolytica,* there is a highly significant difference between the two, when the *t* test is applied.

Inasmuch as *Entamoeba hartmanni* is morphologically distinguishable from *E. histolytica,* it is no longer advisable to use the term «small race of *E. histolytica*». Nor is it possible to use 10 μ as a dividing line, for some *E. histolytica* are

less than 10 $\mu$ in diameter and occasionally trophozoites of *E. hartmanni* may slightly exceed 10 $\mu$ in diameter. And, as shown by Freedman and Elsdon-Dew (1958), *E. hartmanni* retains its individuality, even when placed in the same culture with *E. histolytica*.

## BIBLIOGRAPHY

Burrows, R. B. — *Amer. J. Hyg.*, **65**: 172-188, 1957.
Freedman, L., and Elsdon-Dew, R. — *Nature*, **181**: 433-434, 1958.

# LABORATORY DIAGNOSIS OF AMEBIASIS

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Although it is now generally recognized that not all persons infected with *Entamoeba histolytica* have clinical amebiasis, only rarely is it justified to diagnose a case of the disease without demonstrating the presence of the parasite in the patient. This requirement places a great responsibility on the laboratory. In many instances, laboratories located in the same area may vary considerably in the number of *E. histolytica* infections reported. Although some of these differences may be due to the type of patients examined, unfortunately they frequently result from differences in interest, training and experience of the technicians and from differences in technical procedures employed. As a result, in many places of the world where amebiasis does not exist as a major disease it does constitute a serious diagnostic problem. It is therefore appropriate at this international congress to review briefly the subject of laboratory diagnosis.

## DIFFERENTIATION OF THE INTESTINAL AMEBAE

At least five species of amebae live in the intestinal tract of man and may be encountered in stool specimens: *E. histolytica, Entamoeba coli, Endolimax nana, Iodamoeba bütschlii,* and *Dientamoeba fragilis.* Only *E. histolytica* is recognized as a pathogen, but it is difficult to

differentiate it from the other species. Furthermore, there is increasing evidence that this species should be differentiated from perhaps a subspecies *E. histolytica hartmanii* (Hoare, 1950) or a separate species *Entamoeba hartmanni* (Burrows, 1957; Freedman and Elsdon-Dew, 1958), which morphologically is quite similar. Occasionally, *Endamoeba polecki* may have to be differentiated from *E. histolytica* (Burrows and Klink, 1955).

Although there are classical descriptions of the amebae upon which the various species are justified (Craig, 1944; Anderson, Bostick and Johnstone, 1953), frequently the organism under the microscope fails to conform. The novice who attempts to identify the species by referring simply to text book descriptions and drawings is likely to make incorrect identification. Unfortunately, authorities do not agree too well on what morphological characteristics constitute sufficient proof that an ameba is *E. histolytica,* particularly in the trophozoite stage (Brooke *et al.,* 1953).

There is considerable overlapping of characteristics so that an organism of one species may at times resemble another. As Rizzotti (1952) has pointed out, the morphology of *E. histolytica* may change during the period of examination to such a degree that it will resemble *E. coli.* Since trophozoites are more subject to all types of variations, they are notoriously difficult to differentiate. Unless all of the classical characteristics are present, as may be the case in acute dysentery, it is generally ill-advised to make a species diagnosis based upon trophozoites alone (Lynch, 1931; Dolkart and Hedges, 1947; Bijlmer, 1948).

Perhaps in the future, identification of intestinal amebae will constitute a more exact science through the utilization of procedures which will give more objective information. The fluorescent antibody technique (Goldman, 1953) and the Feulgen staining method (Pan and Geiman, 1955) are promising procedures, but at the present they are research tools rather than diagnostic methods. Although Baud and Morard (1951) have used the measurement of birefringence with the polarizing microscope to differentiate species of *Amoeba,* apparently the technique has not been applied to the study of parasitic amebae.

## COLLECTION OF STOOL SPECIMENS

Ideally, the stool specimen should be passed in close proximity to the laboratory and examined immediately while still warm. In view of the fact that *E. histolytica* is parasitic in a warm blooded host, it would appear logical to keep the specimen artificially warm until it is examined. On the contrary, it has been observed (Tsuchiya, 1945; Gurevitch and Delightish, 1947) that to keep the specimen at body temperature will hasten the death of the trophozoites. Since trophozoites do not multiply or encyst outside the body, they are destined to die and degenerate. Since this is hastened by elevated temperatures, if a fresh specimen cannot be examined immediately, it should be kept at room temperature or in the refrigerator rather than artificially warmed.

Many diagnostic laboratories, particularly those of health departments which are located some distance from the patients, have not been in a position to assist physicians with cases of acute amebiasis. The person with acute amebic dysentery passes only the trophozoites. If such a specimen is delayed in reaching the laboratory, the etiologic agent, even though originally present, is usually unrecognizable or completely disintegrated by the time it is examined. In 1949 Brooke and Goldman described a method of preserving intestinal protozoa in vials of polyvinyl alcohol (PVA) fixative, a solution containing a water soluble resin and Schaudinn's fixative. After a specimen has been mixed in a vial with PVA-fixative, trophozoites that are present will remain suitable for staining and identification for several months. The effectiveness of PVA-fixative in preserving the intestinal protozoa, particularly in the trophozoite stage, has been demonstrated in several studies by different investigators (Young *et al.*, 1951; Goldman and Brooke, 1953; Regonesi *et al.*, 1954; Buonomini and Braccini, 1954; Brooke *et al.*, 1955; Artigas, 1955; Brooke *et al.*, 1956). A two vial method of shipping stool specimens which enables the laboratory to recover all diagnostic stages of the intestinal parasites has been advocated. One vial of the kit contains PVA-fixative while the other is empty. In the United States, a number of state public health department laboratories have been providing shipment kits utilizing this procedure with good results (Kelley, 1952; Harper, Little and Damon,

1957). With the demonstration that an effective concentration procedure can be performed on formalin preserved specimens (Ritchie, 1948), laboratories have started substituting in the kit a vial containing 5 % to 10 % formalin for the empty one as originally proposed.

Harper, Little and Marshall (1957) have reported the effectiveness of a kit for collecting specimens in merthiolate-iodine-formalin solution (Sapero and Lawless, 1953). They have recommended it, particularly for survey work, when only one technique, MIF concentration (Blagg *et al.*, 1955), is performed. This procedure combined with stained films of aliquots preserved in PVA-fixative was the most effective combination, particularly for revealing *E. histolytica*. Although the writer prescribes to these conclusions, the use of MIF may complicate the collection too much for routine use by a diagnostic laboratory. In comparative observations at the Communicable Disease Center, concentrations of MIF preserved specimens have not given significantly better results than the concentration on portions of the same specimens preserved simply in 5 % to 10 % formalin. In employing MIF as a preservative it is necessary to add the iodine solution to the MF solution just prior to the collection of the specimen. This is not a complicated manipulation, but many patients have difficulty following the simplest directions unless individually instructed. Furthermore, the iodine solution deteriorates within a few weeks, while formalin solutions last indefinitely. Many of the cysts and some trophozoites can be identified in wet mount preparations (MIF and others), but it is always advisable to confirm the identification of trophozoites in permanently stained films. Therefore, although MIF can be substituted for formalin, it is not essential in the collection of stool specimens. Furthermore, as Harper and her co-workers (1957) state, it is important to include in the collection kit a vial of PVA-fixative in order that stained films can be prepared.

It is not possible to state how many stool specimens have to be examined to determine if a person is free of infection with *E. histolytica*. However, the investigation of Andrews (1934), Sawitz and Faust (1942) and others have demonstrated the value of multiple specimens in increasing the possibility of finding organisms. This is due, in part, to the limitations of techniques employed and the intermittent passing of organisms from the host. In any event, the physician receiving a

report of «no parasites found» on a single specimen should realize that this does not exclude the possibility of this patient being infected.

As a practical routine, at least two or three normally passed stool specimens, preferably spaced several days apart, should be examined before resorting to catharsis or sigmoidoscopy. Normally passed specimens are more likely to contain cysts which can be identified with greater reliability than trophozoites (Bijlmer, 1948; Brooke *et al.*, 1953). Andrews (1934) has shown that the possibility of finding organisms in a single specimen is increased by purgation. The patient receiving catharsis should be directed to collect all bowel movements in separate boxes numbered in series and to deliver them immediately to the laboratory. Each post-cathartic specimen should be examined for *cysts* as well as trophozoites.

## COLLECTION OF SIGMOIDOSCOPIC MATERIAL

If a patient has had a recent bowel movement when he reports to the clinic, sigmoidoscopy may be performed immediately. If a spontaneous bowel movement does not occur, a cathartic may be given. In this case, it is necessary to delay sigmoidoscopy 2 or 3 hours after purgation. A cleansing enema is not advisable since it may remove organisms from the area which can be viewed through the sigmoidoscope. In order to maintain systematic and uniform records of examinations, the physician may wish to record his observations on a form similar to the one used in the study of dysentery cases in a prisioner-of-war camp in Korea (Brooke, Mason and Hardy, 1959). Such a form encourages the recording of *objective* observations which can be compared and analyzed with greater accuracy.

A cotton swab should *not* be used to obtain material for microscopic examination. A serologic pipette (1 ml) with a small rubber buld may be used to aspirate material from visible lesions and the mucosa. Likewise, a curette or Volkman spoon may be used to gently scrape pathologic areas or the mucosal wall. If desired, rectal punch biopsies can be performed. Manson-Bahr and Muggleton (1957) have described the appearance of unstained biopsy specimens in different types of colitis; and recently Juniper and co-workers (1958) reported

on the study of 14 amebic colitis cases by stained sections of biopsied material.

## COLLECTION OF LIVER ABSCESS ASPIRATES

Liver abscesses suspected of being caused by amebae may be aspirated in an attempt to confirm the diagnosis or as an aid to therapy. Frequently, *E. histolytica* is not demonstrated in such aspirates. Elsdon-Dew (1958) relates a procedure which has proved highly successful in revealing amebae in his studies. During aspiration, various portions of the exudate are kept separate. Contrary to the published literature, the first portion withdrawn is not the color of «anchovy paste», but is yellowish-white. Amebae are never found in this fraction. Later portions which are reddish in color are more likely to contain organisms. Contraction of the abscess is believed to release amebae located in the peripheral areas. The enzyme, streptodornase, is added to the thick pus (10 units per milliliter of pus) and the mixture incubated for 30 minutes with repeated shaking. This procedure frees the amebae from the coagulum, permitting their demonstration in direct wet mounts or after cultivation in media preconditioned with *Clostridium welchii*.

## DIRECT METHODS OF EXAMINATION

In the diagnosis of amebiasis there are four general types of direct examinations that can be performed on stool specimens: wet mount examinations, concentration procedures, permanently stained preparations, and cultivation.

*Temporary wet mounts* made directly from stool specimens require the least equipment and are the simplest to prepare. Using various solutions, they will generally permit the identification of all types and stages of organisms which are encountered. Three general types of solutions are used in the preparation of the temporary wet mounts for the identification of the intestinal protozoa: physiological saline solution for both trophozoites and cysts, iodine solution (Dobell's; or Lugol's diluted 1:5) for cysts, and some other staining solution for trophozoites. Various staining solutions have been advocated to assist with the identification of amebic trophozoites. Quensel's solu-

tion (Svensson, 1935; Mackie, Hunter and Worth, 1945) has had perhaps the widest acceptance.

Nair (1953) has shown that the visibility of the nucleus of *E. histolytica* trophozoites depends on the pH of the suspending solution. He found that the optimal pH with an acetate buffer was 4.0-4.8 and with citrate or phthalate buffers 2.0.-2.5. According to this information, the technician need not have the more complicated staining solutions, but simply use methylene blue, pyronin, and other available stains in a buffered solution of desired pH.

In the diagnosis of amebiasis, *concentration techniques* are useful in revealing light infections where cysts are present, but as yet there is no satisfactory method for concentrating trophozoites. The zinc sulfate flotation technique (Faust *et al.*, 1938) is an effective concentration procedure for unpreserved specimens. It has been utilized in numerous laboratories with slight modifications with good results.

The formalin ether sedimentation technique (Ritchie, 1948) is likewise effective in concentrating both protozoan cysts and helminth eggs from unpreserved specimens and in addition can be performed satisfactorily on formalin preserved specimens. In comparative studies with the zinc sulfate flotation procedure the formalin ether sedimentation (FE) technique has been considered to be the better (Ritchie, Pan and Hunter, 1952, 1953). The MIF ether concentration (MIFC) technique proposed by Blagg *et al.* (1955) is essentially the Ritchie technique with the substitution of MIF stain for formalin. It can be performed on either fresh specimens or those preserved in the MIF solution.

Various authorities have emphasized the importance of using *permanently stained fecal preparations* for the demonstration and accurate identification of intestinal protozoa (Meyer and Johnstone, 1935; Goldman and Brooke, 1953; Anderson *et al.*, 1953; Brooke *et al.*, 1954, and others). In temporary wet mounts, unless indisputably typical characteristics of *E. histolytica* are observed (preferably in the cystic state), the identification should be considered only as tentative. Confirmation in permanently stained slides is essential for accurate diagnostic work. In addition to aiding in the confirmation of questionable organisms permanently stained fecal preparations may reveal organisms not found by other techniques. The increase is due primarily to the finding of small organisms (Wenrich *et al.*, 1935; Faust

*et al.*, 1939; Goldman and Brooke, 1953, and others). Since the procedure need not be time consuming, it is strongly recommended to stain all specimens found to contain amebae or questionable organisms.

For several decades, the Heidenhain's iron hematoxylin procedure has been preferred by many for staining fecal smears. Although it may take a day or two to complete, frequently it can be incorporated into a busy laboratory regimen more easily than a shorter procedure which requires constant attention. A number of simpler staining techniques which can be completed in less than an hour have been developed (e.g., Tompkins and Miller, 1947, and Wheatley, 1951). In a comparative study, Markell (1956) has shown Wheatley's trichrome method to be very satisfactory for diagnostic purposes.

*Cultivation* can be employed to advantage with *fresh* specimens if there are adequate facilities, time and interest, but it is not a recommended procedure for laboratories which receive specimens by mail (Norman and Brooke, 1955*a*). Several media are available *e.g.*, liver extract (Cleveland and Colliers, 1930); modified Boeck and Drbohlav's, or LER (Boeck and Drbohlav, 1925; Reardon and Rees, 1939); egg yolk infusion (Balamuth, 1946); and alcohol extract (Nelson, 1947). However, rather than using several media, it is generally better to select a single medium and to become proficient in its use. With most media it is generally advisable to add small quantities of penicillin and streptomycin prior to inoculation in order to suppress bacterial multiplication (Spingarn and Edleman, 1952; Norman and Brooke, 1955a).

Although *E. histolytica* tends to culture more easily than the other species, there is no satisfactory differential medium that will isolate only the pathogen from stool specimens. For this reason, after the amebae have been successfully established in culture there is still the necessity of differentiating the species on morphological characteristics. The full effectiveness of cultivation as a diagnostic procedure will not be realized unless permanently stained preparations are made from the sediments. Norman and Brooke (1955*b*) increased the number of positive identifications from 212 to 391 by examining permanently stained culture sediments preserved in PVA-fixative.

## INDIRECT METHODS OF DIAGNOSIS

Since there are occasions when it is impossible to demonstrate the presence of *E. histolytica* in patients suffering from amebiasis, there is a great need for an effective indirect diagnostic (serologic) test. This is particularly true in cases of extra-intestinal amebiasis. Unfortunately, at the present time serology has little practical assistance to offer. The complement fixation first proposed by Craig in 1929 is considered to be of questionable assistance in intestinal amebiasis (Dolkart *et al.,* 1951; Lippi *et al.,* 1952; Buchman *et al.,* 1952). Although it has been shown that it has value in confirming extra-intestinal involvement (Terry and Bozicevich, 1948; Hussey and Brown, 1950; Haedicke, 1951; McDearman and Dunam, 1952; Elsdon-Dew and Maddison, 1952; Terry, 1955; Kenney *et al.,* 1955), satisfactory antigens for the complement fixation test are difficult to prepare or are not readily available. Recently an immobilization test Cole and Kent, 1953), a phagocytic index test (Shaffer and Balsam, 1954; Shaffer and Ansfield, 1956) and a precipitin procedure (Moan, 1957) have been proposed, but none of these tests has had sufficient evaluation to be recommended at this time. A fluorescence inhibition test similar to the one for toxoplasmosis (Goldman, 1957) is being investigated at the Communicable Disease Center.

## SELECTION OF TECHNIQUES FOR ROUTINE USE

Since many techniques and procedures have been advocated for the laboratory confirmation of intestinal amebiasis, some selection usually has to be made in establishing a laboratory regimen for this purpose. There are values and limitations to each type of examination described above and all are subject to technical errors on the part of the person performing them. In general, the more types of examinations that are included in the laboratory routine, the greater the number of infections that will be discovered. However, from a practical standpoint the laboratory director must decide if the increase in positive findings warrants the additional time and expense of performing the added techniques. In practice it would seem advisable to select certain

procedures to be used routinely and reserve others as supplementary techniques for special occasions. In selecting the procedures to be used routinely, the laboratory should include methods which will enable the recovery and identification of different types and stages of organisms which may be present. The availability of the patient and whether specimens are received fresh or through the mail will also influence the choice of techniques. Recently the writer suggested a number of laboratory regimens for various situations (Brooke, 1958). On the occasion of this international meeting, when we are concerned

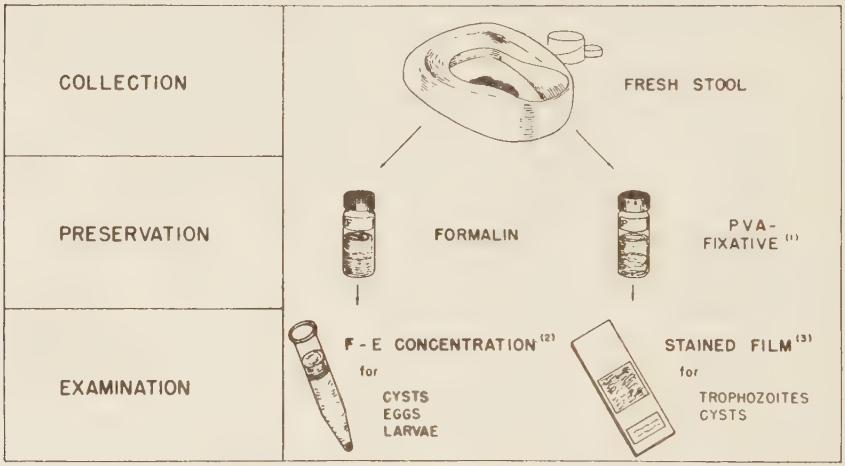


Fig. 1 — Parasitological examination of stool specimens

with information on the geographical distribution of *E. histolytica* in the world, it is well to limit our consideration to *suggested procedures for prevalence surveys.*

In conducting surveys to determine the prevalence of *E. histolytica* and other intestinal parasites it is important that the procedures be able to recover all stages and types of parasites and, at the same time, be limited enough to be practical. The formalin ether concentration technique and permanently stained films preformed on fecal samples preserved respectively in formalin and PVA-fixative (Fig. 1) have proved to be satisfactory in a number of surveys conducted by the Communicable Disease Center in cooperation with other organi-

zations (Brooke *et al.*, 1955; Le Maistre *et al.*, 1956; Brooke *et al.*, 1956; Brooke *et al.*, 1958).

The only part of the operation that has to be performed in the field is the preservation of the specimens as they are collected. Since this can be accomplished with unskilled personnel, and with practically no equipment, the expense of the field aspects of the survey can be greatly reduced. However, if desired, various segments of the laboratory work can be conducted in the field, depending upon the personnel and facilities available. If it is desirable to have a preliminary estimate of the prevalence during the field investigation, the formalin-ether concentrations can be completed and examined. The portions of the specimen preserved in PVA-fixative can be examined later in the central laboratory. On other occasions it may be desirable to *a*) concentrate the specimen and ship the sediments in formalin to the central laboratory for examination, and *b*) prepare the dried films of PVA fixed specimens for staining and examination at a later time.

If it is necessary to reduce the laboratory work involved in the survey, the examination of the stained PVA films can be restricted to those specimens which revealed parasites or questionable organisms in the concentrations and to those specimens which were observed to be soft and/or contain blood and mucus when collected. These abnormalities must be recorded in the field during the collection of the specimens.

## EVALUATION OF LABORATORY PERFORMANCE

Any laboratory procedure employed for the direct demonstration of amebae is only as reliable as the microscopist who examines the preparation. Many technicians have not been training to differentiate the harmless amebae from *E. histolytica* and too frequently, perhaps for fear of missing the pathogens, any amebae found may be reported as a pathogenic species. On the other hand, owing to the lack of interest or ability, positive cases may be completely overlooked. It is imperative that all persons attempting to identify the intestinal protozoa should receive intensive training and supervised experience before being relied upon for independent work. In themselves, no

academic degree or certificate should be taken as proof of proficiency in this field. The physician or laboratory director employing technical assistants for the identification of intestinal amebae should evaluate their performance before accepting their results.

The most effective means of assuring a high level of proficiency in the laboratory is to have check specimens examined regularly as «unknowns». A few years ago, in the United States, the state public health laboratories engaged in a parasitological evaluation which emphasized the identification of the intestinal amebae (Brooke and Hogan, 1952). Since then a number of state laboratories have established similar intra-state evaluations of local, public and private laboratories (see personal communications). Individuals attending this international congress could help greatly to eliminate amebiasis as a diagnostic problem in their area of the world by urging their public health agency or other appropriate organization to establish continuing evaluation programs for the mutual benefit of the local microscopists and the patients they examine.

## BIBLIOGRAPHY

ANDERSON, H. H., BOSTICK, W. L., and JOHNSTONE, H. G. — *Amebiasis — Pathology, diagnosis and chemotherapy*. Charles C. Thomas, Publisher, Springfield, Illinois. xiii+431 pages, 1953.

ANDREWS, J. — «The diagnosis of intestinal protozoa from purges and normally-passed stools», *J. Parasitol.*, **20**: 253-254, 1934.

ARTIGAS, J. — «Técnica de tinción por hematoxilina molíbdica para protozoos en frotis de deposiciones preservadas en fijador con alcohol polivinílico», *Bol. Chil. de Parasitol.*, **10** (3): 57-58, 1955.

BALAMUTH, W. — «Improved egg yolk infusion for cultivation of *Entamoeba histolytica* and other intestinal protozoa», *Am. J. Clin. Path.*, **16**: 380, 1946.

BAUD, C. A., and MORARD, J. C. — «Études des amibes de la collection Penard au microscope polarisant», *Arch. des Sci.*, **4** (6): 415-417, 1951.

BIJLMER, J. — «On the recovery of protozoa and eggs of some species of helminths in human feces», *J. Parasitol.*, **34**: 101-107, 1948.

BLAGG, W., SCHLOEGEL, E. L., MANSOUR, N. S., and KHALAF, G. I. — «A new concentration technic for the demonstration of protozoa and helminth eggs in feces», *Am. J. Trop. Med. & Hyg.*, **4**: 23-28, 1955.

BOECK, W. C., and DRBOHLAV, J. — «The cultivation of *Endamoeba histolytica*», *Am. J. Hyg.*, **5**: 371-407, 1925.

BROOKE, M. M. — *Amebiasis — Methods in Laboratory Diagnosis.* Communicable Disease Center publication. Dept. of Health, Education and Welfare, Public Health Service. iii+67 pages, 1958.

BROOKE, M. M., DONALDSON, A. W., and BROWN, E. — «An amebiasis survey in a veterans administration hospital, Chamblee, Georgia, with comparison of technics», *Am. J. Trop. Med. & Hyg.*, **3** (4): 615-620, 1954.

BROOKE, M. M., and GOLDMAN, M. — «Polyvinyl alcohol-fixative as a preservative and adhesive for protozoa in dysenteric stools and other liquid materials», *J. Lab. Clin. Med.*, **34** (11): 1554-1560, 1949.

BROOKE, M. M., and HOGAN, R. B. — «An evaluation of enteric parasitology performed in state laboratories», *Pub. Health Rep.*, **67**: 1237-1249, 1952.

BROOKE, M. M., MASON, R., and HARDY, A. V. — «A form for recording sigmoidoscopic examinations of dysentery cases», *U.S.A. Arm. Forces Med. J.*, **10** (10): 1174-1184, 1959.

BROOKE, M. M., MELVIN, D. M., SAPPENFIELD, R., PAYNE, F., CARTER, F. R. N., OFFUTT, A. C., and FRYE, W. W. — «Studies of a waterborne outbreak of amebiasis, South Bend, Indiana. III. Investigation of family contacts», *Am. J. Hyg.*, **62**: 214-226, 1955.

BROOKE, M. M., OTTO, G., BRADY, F., FAUST, E. C., MACKIE, T. T., and MOST, H. — «An analysis of a memorandum on the diagnosis of amebiasis», *Am. J. Trop. Med. & Hyg.*, **2**: 593-612, 1953.

BROOKE, M. M., SWARTZWELDER, C., PAYNE, F. J., WEINSTEIN, P., and FRYE, W. W. — «Intestinal parasite survey of Korean prisoner-of-war camp», *U.S. Armed Forces Med. J.*, **7**: 708-714, 1956.

BUCHMAN, E., KULLMAN, H. J., and MARGONIS, G. F. — «Evaluation of the complement fixation test in amebiasis», *Gastroenterology*, **21** (3): 391-399, 1952.

BUONOMINI, G., and BRACCINI, L. — «Su la diagnosi parassitologica di amebiasi. Nota II. Proposta di uno schema di indagini ai fini di una corretta diagnosi di *E. histolytica*», *Revista Ital. d'Igiene*, **14** (1-2): 1954.

BURROWS, R. B. — «*Endamoeba hartmanni*», *Am. J. Hyg.*, **65**: 172-188, 1957.

BURROWS, R. B., and KLINK, G. E. — «*Endamoeba polecki* infections in man», *Am. J. Hyg.*, **62** (2): 156-167, 1955.

CLEVELAND, L. R., and COLLIER, J. — «Various improvements in the cultivation of *Entamoeba histolytica*», *Am. J. Hyg.*, **12**: 606-613, 1930.

COLE, B. A., and KENT, J. F. — «Immobilization of *Endamoeba histolytica* in vitro by antiserum produced in the rabbit», *Proc. Soc. Exper. Biol. & Med.*, **83**: 811-814, 1953.

CRAIG, C. F. — *The etiology, diagnosis and treatment of amebiasis.* The Williams & Wilkins Company, Publisher, Baltimore, Maryland. viii+332 pages, 1944.

DOLKART, R. E., HALPERN, B., and CULLEN, J. — «The diagnosis of amebiasis; the role of the complement fixation test, and the incidence of the disease in the Chicago area», *J. Lab. & Clin. Med.*, **38** (5): 804, 1951.

DOLKART, R. E., and HEDGES, R. N. — «The present incidence of intestinal infestation with *E. histolytica* and other protozoa in the Chicago area», *Gastroenterology*, **9** (2): 170-176, 1947.

ELSDON-DEW, R. — Personal communication, 1958.

ELSDON-DEW, R., and MADDISON, S. E. — «Amoebic complement-fixation reaction», *J. Trop. Med. & Hyg.*, **55** (9): 208-211, 1952.

FAUST, E. C., D'ANTONI, J. S., ODUM, V., MILLER, M. J., PERES, C., SAWITZ, W., THOMEN, L. F., TOBIE, J. E., and WALKER, J. H. — «A critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in feces», *Am. J. Trop. Med.*, **18**: 169-183, 1938.

FAUST, E. C., SAWITZ, W., TOBIE, J., ODOM, V., PERES, C., and LINCICOME, D. R. — «Comparative efficiency of various technics for the diagnosis of protozoa and helminths in feces», *J. Parasitol.*, **25**: 241-262, 1939.

FREEDMAN, L., and ELSDON-DEW, R. — «Size variation in *Entamoeba histolytica*», *Nature*, **181**: 433-434, 1958.

GOLDMAN, M. — «Staining *Toxoplasma gondii* with fluorescein-labelled antibody. II. A new serologic test for antibodies to toxoplasma based upon inhibition of specific staining», *J. Exper. Med.*, **105** (6): 557-573, 1957.

GOLDMAN, M. — «Cytochemical differentiation of *Endamoeba histolytica* and *Endamoeba coli* by means of fluorescent antibody», *Am. J. Hyg.*, **58**: 319-328, 1953.

GOLDMAN, M., and BROOKE, M. M. — «Protozoans in stools unpreserved and preserved in PVA-fixative», *Pub. Health Rep.*, **68** (7): 703-706, 1953.

GUREVITCH, J., and DELIGHTISH, J. — «Survival time of *Endamoeba histolytica* in feces», *Harefuah*, **32**: 60, 1947.

HAEDICKE, T. A. — «Clinical value of the complement fixation test for hepatic amebiasis», *M. Ann. District of Columbia*, **20**: 415-420, 1951.

HARPER, K., LITTLE, M. D., and DAMON, S. R. — «Advantages of the PVA-fixative two-bottle stool collection technic in the detection and identification of intestinal parasites», *Pub. Health Lab.*, **15**: 96, 1957.

HARPER, K., LITTLE, M. D., and MARSHALL, A. L. — «Comparison of stool collection techniques in amebiasis investigations», *Pub. Health. Rep.*, **72** (11): 1031-1037, 1957.

HOARE, C. A. — *Handbook of medical protozoology*. Williams and Wilkins Co., Publishers, Baltimore, Maryland. XV+334 pages, 1950.

HUSSEY, K. L., and BROWN, H. W. — «The complement fixation test for hepatic amebiasis», *Am. J. Trop. Med.*, **30**: 147-157, 1950.

JUNIPER, K., JR., STEELE, V. W., and CHESTER, C. L. — «Rectal biopsy in the diagnosis of amebic colitis», *South. M. J.*, **51**: 545-553, 1958.

KELLEY, G. W. — «Evaluation of the PVA method of fixing ameba trophozoites for shipment to diagnostic laboratories», *Nebraska M. J.*, **37** (11): 367-368, 1952.

KENNEY, M., WOLF, J., and TATZ, J. S. — «The evaluation of the amebic complement-fixation test», *Am. Pract. & Digest. Treat.*, **6** (8): 1201-1204, 1955.

LE MAISTRE, C. A., SAPPENFIELD, R., CULBERTSON, C., CARTER, F. R. N., OFFUTT, A., BLACK, H., and BROOKE, M. M. — «Studies of a water-borne outbreak of amebiasis, South Bend, Indiana. I. Epidemiological aspects», *Am. J. Hyg.*, **64** (1): 30-45, 1956.

LIPPI, M., CAPOCACCIA, L., and CAO-PINNA, M. — «Richerche sulla reazione di devia-

zione del complemento nell'amebiasi», *Arch. Ital. Sci. Med. Trop. Parassit.*, **11**: 605-618, 1952.

LYNCH, K. M. — «The prevalence of intestinal amebiasis», *Am. J. Clin. Path.*, **1**: 265-267, 1931.

MCDEARMAN, S. C., and DUNHAM, W. B. — «Complement fixation tests as an aid in the differential diagnosis of extra-intestinal amebiasis», *Am. J. Trop. Med. & Hyg.*, **1** (2): 182-188, 1952.

MACKIE, T. T., HUNTER, G. W., and WORTH, C. B. — *Manual of tropical medicine*. Saunders, Publisher, Philadelphia, Pennsylvania, XIX+727 pages, 1945.

MANSON-BAHR, P., and MUGGLETON, W. J. — «Rectal biopsy as an aid to the diagnosis of amoebic dysentery and allied diseases of the colon», *Lancet*, **6972**: 763-765, 1957.

MARKELL, E. K. — «A comparison of three staining techniques for protozoan parasites as applied to PVA-preserved fecal specimens», *J. Parasitol.*, **42** (5): 478, 1956.

MEYER, K. F., and JOHNSTONE, H. G. — «Laboratory diagnosis of amebiasis», *Am. J. Pub. Health*, **25**: 405-414, 1935.

MOAN, J. C. — «The serological diagnosis of amebiasis by means of the precipitin test», *Am. J. Trop. Med. & Hyg.*, **6** (3): 499-513, 1957.

NAIR, C. P. — «Rapid staining of intestinal amoebae on wet mounts», *Nature*, **172**: 1051, 1953.

NELSON, E. C. — «Alcoholic extract medium for the diagnosis and cultivation of *Endamoeba histolytica*», *Am. J. Trop. Med.*, **27**: 525, 1947.

NORMAN, L., and BROOKE, M. M. — «The use of penicillin and streptomycin in the routine cultivation of amebae from fecal specimens», *Am. J. Trop. Med. & Hyg.*, **4** (3): 472-478, 1955*a*.

NORMAN, L., and BROOKE, M. M. — «The effectiveness of the PVA-fixative technique in revealing intestinal amebae in diagnostic cultures», *Am. J. Trop. Med. & Hyg.*, **4** (3): 479-482, 1955*b*.

PAN, C., and GEIMAN, Q. M. — «Comparative studies of intestinal amebae. I. Distributions and cyclic changes of the nucleic acids in *Endamoeba histolytica* and *Endamoeba coli*», *Am. J. Hyg.*, **62** (1): 66-79, 1955.

Personal communications: BODILY, H. L. (Calif. State Pub. Health Lab.), FRASER, R. S. (Utah State Pub. Health Lab.), HARDY, A. V. (Fla. State Pub. Health Lab.), HAUSER, G. H. (La. State Pub. Health Lab.), IRONS, J. V. (Texas State Pub. Health Lab.), KLOTZ, A. W. (Idaho State Pub. Health Lab.), PERRY, C. A. (Maryland State Pub. Health Lab.).

REARDON, L. V., and REES, C. W. — «The cultivation of *Endamoeba histolytica* without serum», *J. Parasitol.*, **25** (Suppl.): 13-14, 1939.

REGONESI, C., MURANDA, M., and ARTIGAS, J. — «Técnica del fijador con alcohol polivinílico en el diagnóstico de la amibiasis y otras enteroparasitosis», *Bol. Chileno Parasitol.*, **9**: 105-109, 1954.

RITCHIE, L. G. — «An ether sedimentation technique for routine stool examinations», *Bull. U.S. Army Med. Dept.*, **8**: 326, 1948.

RITCHIE, L. W., PAN, C., and HUNTER, G. W., III — «A comparison of the zinc sulfate and the MGL (formalin-ether) technics», *J. Parasitol.*, **38** (Suppl.): 16, 1952.

RITCHIE, L. S., PAN, C., and HUNTER, G. W., III — «A comparison of the zinc sulfate and the formalin-ether (406th MGL) technic», *Med. Bull. U.S. Army Far East*, **1**: 111-113, 1953.

RIZZOTTI, G. — «Le caratteristiche morfologiche dei trofozoiti di *Entamoeba histolytica* nelle feci umane, loro significato biologico e loro importanza pratica», *Arch. Ital. Sci. Med. Trop. Parassit.*, **33** (10): 551-560, 1952.

SAPERO, J. J., and LAWLESS, D. K. — «The «MIF» stain-preservation technic for the identification of intestinal protozoa», *Am. J. Trop. Med. & Hyg.*, **2**: 613-619, 1953.

SAWITZ, W. G., and FAUST, E. C. — «The probability of detecting intestinal protozoa by successive stool examinations», *Am. J. Trop. Med.*, **22**: 131-136, 1942.

SHAFFER, J. G., and ANSFIELD, J. — «The effect of rabbit antisera on the ability of *Entamoeba histolytica* to phagocytose red blood cells», *Am. J. Trop. Med. & Hyg.*, **5** (1): 53-61, 1956.

SHAFFER, J. G., and BALSAM, T. — «Ability of *Endamoeba histolytica* to phagocytose red blood cells», *Proc. Soc. Exper. Biol. & Med.*, **85**: 21-24, 1954.

SPINGARN, C. L., and EDELMAN, M. H. — «Further observations on the use of streptomycin and penicillin in the cultivation of *Endamoeba histolytica* from stools», *Am. J. Trop. Med. & Hyg.*, **1**: 412-416, 1952.

SVENSSON, R. — «Studies on human intestinal protozoa», *Acta Med. Scandinav. Suppl.*, LXX, vi+115 pages, 1935.

TSUCHIYA, H. — «Survival time of trophozoites of *Endamoeba histolytica* and its practical significance in diagnosis», *Am. J. Trop. Med.*, **25**: 277-279, 1945.

TERRY, L. I. — «Clinical considerations of amebiasis», *Am. J. Gastroenterology*, **23**: 325-328, 1955.

TERRY, L. I., and BOZICEVICH, J. — «The importance of the complement fixation test in amebic hepatitis», *South. M. J.*, **41**: 691-702, 1948.

TOMPKINS, V. N., and MILLER, J. K. — «Staining intestinal protozoa with iron-hematoxylin-phosphotungstic acid», *Am. J. Clin. Path.*, **17**: 755-757, 1947.

WENRICH, D. H., STABLER, R. M., and ARNETT, J. H. — «*Endamoeba histolytica* and other intestinal protozoa in 1,060 college freshmen», *Am. J. Trop. Med.*, **15**: 331-345, 1935.

WHEATLEY, W. B. — «A rapid staining procedure of intestinal amoebae and flagellates», *Am. J. Clin. Path.*, **21**: 990-991, 1951.

YOUNG, V. M., FELSENFELD, O., SHLAES, W. H., YOSHIMURA, T., and STEIGMANN, F. — «A study of laboratory methods for diagnosing *Endamoeba histolytica* and their application to 5,048 persons from the Chicago area», *Am. J. Digest. Dis.*, **18** (4): 126-130, 1951.

# THE SEROLOGY OF AMOEBIASIS

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For the serological diagnosis of amebiasis several methods have been proposed. Wagner in 1924 (1, 2) used the precipitin test for diagnosing amebiasis in humans and cats. The antigen was prepared by extracting ulcerated areas from the colon of experimentally infected cats. Apparently, the test did not prove successful.

The latest test reported is one described by Moan (3) using an extract of cryolysed trophozoites. The author calls this test a precipitin test, but it should be regarded as a flocculation test. We were not able to obtain the excellent results reported by Moan.

Craig (4) prepared an alcoholic extract as an antigen and employed it in a complement-fixation test. Although this technique detected antibodies, the antigen was not as specific as desirable. The method to be described yielded more potent antigen than the one employed by Craig.

The following is a short description of the method employed in our laboratory.

## PREPARATION OF AMEBAE ANTIGEN

Two methods of preparing amebae antigen have been used by us. In the first, and older method, 25 ml of whole egg medium was placed into a 125 ml Erlenmyer flask. The flask was placed at an angle and the egg medium was inspissated. The slant was overlaid with 50 ml of autoclaved Locke's solution containing 50 mg of dry sterilized

rice flour. The ingredients were autoclaved. The flasks were seeded with an inoculum of *Endamoeba histolytica* trophozoites containing a species of bacteria (a *Clostridium*) which has been designated by Rees (5) as organism *t*. After a 72 hour incubation at 37°C the flasks were harvested. The harvest containing amebae, rice flour and egg

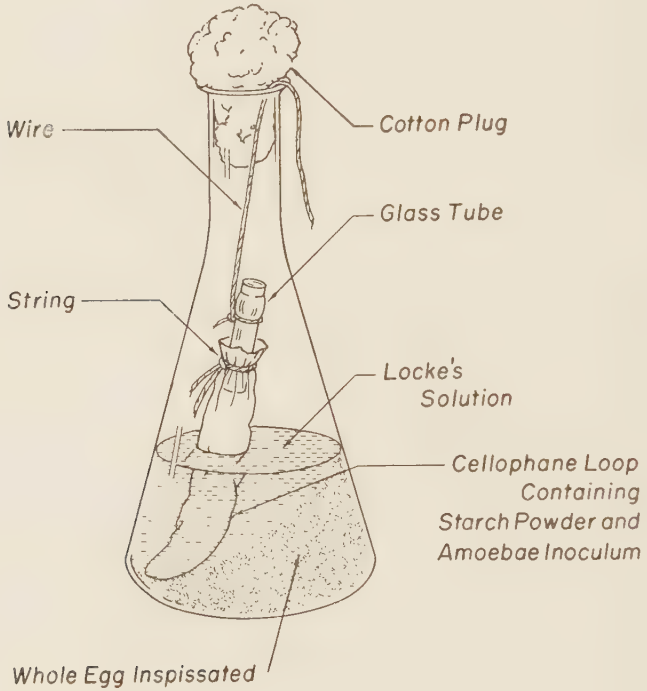


Fig. 1

medium particles was centrifuged for 5 minutes at 2,000 rpm. The sediment was resuspended and washed twice with physiological saline. The resulting sediment was suspended in 10 ml of physiological saline and repeatedly cryolyzed until the amebae were disintegrated. The suspension was next centrifuged for 30 minutes at 15,000 rpm and the supernate used as antigen.

In the second method of preparing the antigen, a loop of dialyzing tubing was placed in a flask (see Fig. 1). 50 mg of rice flour and

10 ml of Locke's solution were placed into the loop. The loop was then seeded with an inoculum of the amebae, as described above. The amebae are allowed to incubate until autolysis has occurred, usually requiring 5 days. The autolysate is centrifuged for 30 minutes at 15,000 rpm and the supernate is employed as antigen. Both antigens are preserved with a 1:1500 final concentration of merthiolate and the antigenic preparations are tested for anticomplementary and hemolytic properties.

## THE COMPLEMENT-FIXATION TEST

The 50 % hemolysis end point complement-fixation method (6) is used. The method quantitatively measures the amount of complement fixed by each sample of serum. There is a great variation in the amount of complement fixed by various sera.

Rabbits were immunized with the final antigen preparation and to an antigen prepared from organism *t* only. Complement-fixation tests were conducted against pooled antisera with the respective antigens. Conversely, tests were conducted with amebae antigen against the pooled serum of rabbits immunized with organism *t*, and with organism *t* antigen against anti-amebae serum, as illustrated in Tables 1 and 2. These tables illustrate the amount of cross reaction with each antigen and antiserum and also show the amount of specific fixation by the respective homologous systems.

The tables also illustrate the amount of organism *t* in the amebae antigen. From the tables it is apparent that an antigen dilution of 1:8 is satisfactory since cross reactions are minimal. It is also shown that the washing and centrifuging removes most of *t* antigen; therefore, a 1:8 dilution of the antigen and a 1:5 dilution of the serum in question may be used. The results reported above were obtained on the antigen prepared by the first method. Antigens prepared by the second method, in which the amebae are allowed to autolyse, contain more *t* antigen. We have found that this does not interfere by giving falsely positive complement-fixation tests. In numerous tests only 1 sample of human serum gave a low titer positive to organism *t* antigen; therefore, the use of organism *t* antigen as a control has been discontinued. It was found that more anticomplementariness

TABLE 1 — *Titration of* E. histolytica *and organism + antigens against organism + antiserum*

| Serum dilution | Antigen dilution | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *E. histolytica* antigen | | | | | Organism + antigen | | | | |
| | 1 : 2 | 1 : 4 | 1 : 8 | 1 : 16 | 1 : 32 | 1 : 2 | 1 : 4 | 1 : 8 | 1 : 16 | 1 : 32 |
| 1 : 2 | ++++ | + | − | − | − | ++++ | ++++ | ++++ | ++++ | ++++ |
| 1 : 4 | +++ | ± | − | − | − | | | | | |
| 1 : 8 | + | − | − | − | − | ++++ | ++++ | ++++ | ++++ | ++++ |
| 1 : 16 | − | − | − | − | − | ++++ | ++++ | ++++ | ++++ | ++++ |
| 1 : 32 | | | | | | ++++ | ++++ | ++++ | ++++ | ++++ |
| 1 : 128 | | | | | | ++ | +++ | +++ | +++ | − |

TABLE 2 — *Titration of* E. histolytica *and organism + antigens against* E. histolytica *antiserum*

| Serum dilution | Antigen dilution | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *E. histolytica* antigen | | | | | Organism + antigen | | | | |
| | 1 : 2 | 1 : 4 | 1 : 8 | 1 : 16 | 1 : 32 | 1 : 2 | 1 : 4 | 1 : 8 | 1 : 16 | 1 : 32 |
| 1 : 2 | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | + | ± | — | — |
| 1 : 4 | ++++ | ++++ | ++++ | ++++ | ++++ | — | — | — | — | — |
| 1 : 8 | ++++ | ++++ | ++++ | ++++ | ++++ | — | — | — | — | — |
| 1 : 16 | ++++ | ++++ | ++++ | ++++ | ++++ | — | — | — | — | — |
| 1 : 64 | ++++ | ++++ | ++++ | ++++ | ++ | | | | | |
| 1 : 128 | ++++ | ++++ | ++++ | ++++ | ++ | | | | | |
| 1 : 512 | +++ | ++++ | ++++ | + | — | | | | | |

obtained with the antigen prepared by the first method was due to the egg medium particles. These particles were very difficult to remove.

## RESULTS

It can be seen in Table 3 that the two strains employed detected more reactors than one of these strains. Strains 103 and 104 were obtained from different sources. The antigens prepared from these differed in specificity. These antigens were tested on 2,412 samples

TABLE 3 — *Shows the number of cases giving either positive or negative complement-fixation reactions with antigens prepared from the two strains of amoebae*

| Antigen prepared from strain 103 | | | Antigen prepared from strain 104 | | |
|---|---|---|---|---|---|
| Positive | Negative | Total | Positive | Negative | Total |
| 699 | 101 | 800 | 699 | 168 | 867 |
| 168 | 1,444 | 1,612 | 101 | 1,444 | 1,545 |
| 867 | 1,545 | 2,412 | 800 | 1,612 | 2,412 |

of serum. This series of sera is not a random sample, but a selected one, from persons suspected of having amebic infection. It can be seen that antigen prepared from strain 103 gave 168 positive complement-fixation tests, which were missed by an antigen prepared from strain 104. Conversely, an antigen from strain 104 gave 101 positive complement-fixation tests missed by an antigen from strain 103.

In support of the contention that a polyvalent antigen is necessary for diagnostic tests, Elsdon-Dew (7) showed that a great difference in pathogenicity of *E. histolytica* exists in various races in South Africa. There is ample evidence that antibodies to amebae are not produced unless there is extensive tissue invasion. As a rule, negative complement-fixation results are obtained on sera from individuals who have mild symptoms. Ruebush, of the National Naval Medical Center at Bethesda, found that the stool examinations on 429 individuals

gave 25 positive findings. Of the 25, 16 showed the presence of small race *E. histolytica*. All 16 gave negative complement-fixation results to antigens prepared from large and small race *E. histolytica* (8). Recently, Freedman and Elsdon-Dew (9) have re-studied the mor-

Fig. 2

phology of the small race *E. histolytica* and they suggest that the small race amebae should be separated in a separate species *Entamoeba hartmanni* Prowazek, 1912.

Shaffer and Ansfield (10) have also shown that antigenic differences exist between strains of *E. histolytica*. These authors immunized

rabbits to various strains of *E. histolytica* and showed that strains varied in their ability to phagocyte red cells when tested against homologous and heterologous immune sera.

The serological diagnosis of amebiasis has its greatest value in extra-intestinal amebic infections. In these instances the stool examination is usually negative. Thus, the physician needs to request various microbiological and serological tests in order to support his suspicion of amebic infection. Terry and Bozicevich (11) and Spicknall *et al.* (12) have shown the value of the complement-fixation test in

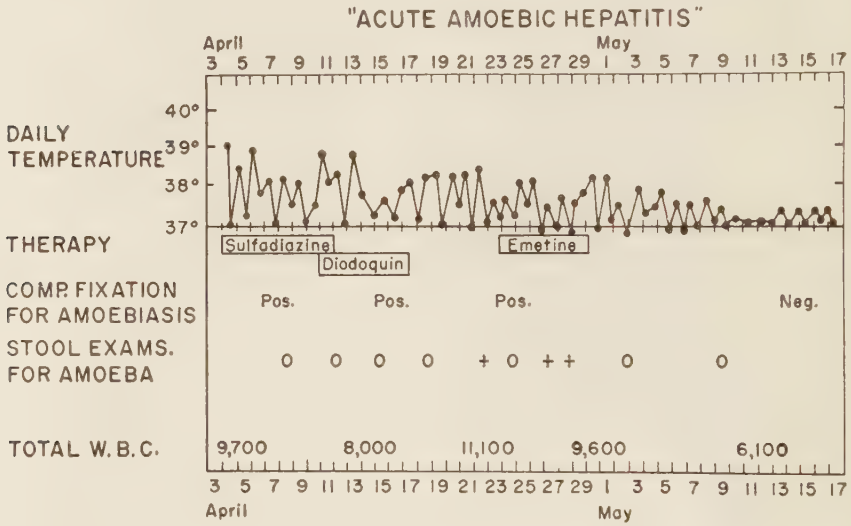


Fig. 3

cases of amebic liver abscess and «amebic hepatitis». Two cases of amebiasis are presented illustrating the value of the complement-fixation test. Fig. 2 illustrates a case in which the X-ray shows an elevation of the right dome of the diaphragm. This individual had pains in the right costal region radiating to the right shoulder. Fig. 3 shows a graphic summary of the hospital course. It can be seen that 2 samples of serum gave positive complement-fixation results before a positive stool finding was obtained. Altogether there were 3 positive complement-fixation tests and 3 positive stool findings. It can also be seen that 14 days after the patient had completed a

course of 7 grains of emetine, the complement-fixation test gave negative results.

Fig. 4 is the X-ray of the chest of a patient who had a hepato-pleuro-bronchial fistula. This patient was seriously ill and had a high

Fig. 4

fever as shown in Fig. 5. Penicillin was administered because of the fever. With a positive complement-fixation test, the patient was given a course of carbarsone. During the carbarsone treatment period, the patient expectorated quantities of reddish-gray material. A second sample of serum was also positive by the complement-fixation test.

The sixth stool examination was positive. At this point emetine therapy was begun. It can be seen that the fever took a downward course. Two additional complement-fixation tests were positive. The patient was discharged and no additional follow-up was done.

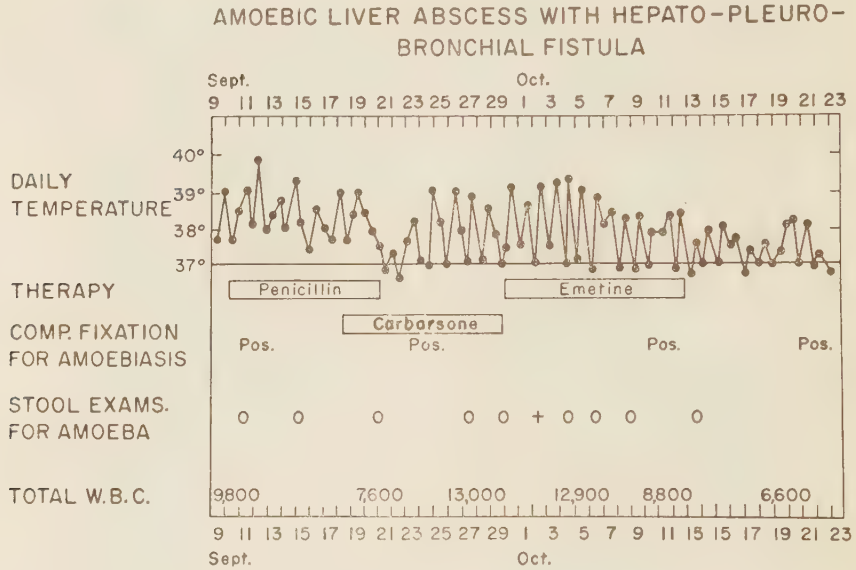


Fig. 5

## DISCUSSION

It must be emphasized that the complement-fixation test should be properly controlled. It is important to know the amount of anti-complementariness of each serum tested. In performing the complement-fixation test, it appears that a polyvalent antigen is essential for greater efficiency. Should there be available an amebic antigen without a contaminating organism, it is conceivable that a more specific and sensitive test may result. It may also be possible to use a monovalent antigen.

The complement-fixation test may have value in following the success of therapy. In the one instance, a negative complement-fixation test was not obtained at the time the patient was discharged.

The second case gave a positive complement-fixation test following successful therapy. It may be that the persistence of antibodies depends somewhat on the extensiveness of tissue invasion of the organism as well as the effectiveness of therapy.

Needless to say that the laboratory results, as with many other laboratory tests, are not pathognomonic for diagnosing amebiasis. One must consider the clinical manifestation and use the laboratory aids as a guide to assist in careful diagnosis of amebiasis.

## BIBLIOGRAPHY


1. Wagner, Edna H. — *Univ. of Calif. Pub. in Zool.,* Berkeley, **26**: 15-20, 1924.
2. Wagner, Edna H., and Thomson, Margaret D. — *Univ. of Calif. Pub. in Zool.,* Berkeley, **26**: 267-280, 1924.
3. Moan, Jeanne C. — *Am. J. Trop. Med. and Hyg.,* **6**: 499-513, 1957.
4. Craig, C. F. — *Am. J. Trop. Med.,* **9**: 277-296, 1929.
5. Rees, Charles W., Bozicevich, John, Reardon, Lucy V., and Jones, Frances — *Am. J. Trop. Med.,* **22**: 581-586, 1942.
6. Bozicevich, John, Hoyem, Helen M., and Walston, Vernal M. — *Public Health Reports,* **61**: 529-534, 1946.
7. Elsdon-Dew, R. — *Am. J. Trop. Med.,* **29**: 337-340, 1949.
8. Bozicevich, John — *Am. J. Trop. Med.,* **30**: 154-157, 1950.
9. Freedman, L., and Elsdon-Dew, R. — *Nature,* **181**: 433-434, 1958.
10. Shaffer, J. C., and Ansfield, J. — *Am. J. Trop. Med. and Hyg.,* **5**: 53-61, 1956.
11. Terry, Luther L., and Bozicevich, John — *South. Med. J.,* **41**: 691-702, 1948.
12. Spicknall, Charles G., Bozicevich, John, Black, Roger L., and Terry, Luther L. — *Gastroenterology,* **32**: 1131-1142, 1957.

## GENERAL DISCUSSION

Dr. John F. Kessel (U.S.A.) — I am pleased to hear the complement-fixation test emphasized again, for I still think serologic tests in amebiasis have merit when antigens of high potency are used. Dr. Backhouse of Sydney, Australia, recently showed me results on a series which he had taken care to correlate with clinical findings, especially sigmoidoscopic examination. Sera from individuals with (1) acute clinical amoebiasis produced more than 90 % positive C-F test results, (2) chronic amoebiasis, about 60 % positives, (3) no manifest clinical symptoms, i.e., «carriers», about 25 % positives.

It is of interest to note how closely these percentages correlate with the findings on virulence of *E. histolytica* by Dr. Neal. His first chart showed that most strains from individuals with acute amoebiasis had a high virulence when tested in rats and that strains from carriers showed a lower percentage of strains with a high virulence. Previous studies by Meleney and Frye using kittens likewise showed most strains from acute cases of amoebiasis to be of high virulence, but an occasional strain from a carrier to possess a high pathogenic index. Dr. Brumpt's *E. dispar* also produced a low pathogenicity in kittens.

The differentiation by Dr. Burrows of *E. hartmanni* from dwarf strains of *E. histolytica* on the basis of a crescentic chromatin mass encrusted on the nuclear membrane of the nucleus is of great interest. Laboratory workers frequently encounter *E. histolytica* showing this structure, not only in dwarf strains, but in large strains as well, e.g., Kofoid's species *dissimilis*. Would Dr. Burrows consider that the genus *E. hartmanni* should be extended to include larger amoebae with this same characteristic or does he draw the line of cysts for this species to be below 10 $\mu$ in diameter?

Sir Philip Manson-Bahr (U.K.) — From a life-long experience of amoebiasis and amoebic dysentery, he would like to congratulate

Dr. Elsdon-Dew, and especially Drs. Burrows & Brooke, on their very helpful studies on the differentiation of non-pathogenic amoebae, resembling *Entamoeba histolytica*. These very minute morphological differences in the structure of the nucleus of *E. hartmanni* can only be appreciated after prolonged staining methods. His own experiences of amoebic dysentery have led him to believe that there is a dearth of pathologists who are proficient in intestinal protozoology, with the result that there are lots of patients who are convinced that they are suffering from intestinal amoebiasis in the absence of any tangible evidence. It can be well said that the *Entamoeba histolytica* more often resides in their heads than in their bowels. He believed that the only safe method of diagnosing intestinal amoebiasis was by taking an exact clinical history and by using all practical methods of making a diagnosis, including proctoscopy, by having the patient on one side and the microscope on the other. As regards his attitude to these non-pathogenic strains *(E. hartmanni)*, what was the clinician to do? Refuse to treat half the number of so-called cases of amoebiasis only to leave them to his rival to treat and presumably cure? Or was he to expect the amoebic cyst on the slide to put up a flag to show to which side it ought to belong? So far all the methods suggested were beyond the range of practical politics. Patients could not, or would not, remain in suspense until a positive diagnosis had been arrived at.

Dr. I. de Carneri (Italy) — Je voudrais dire deux mots à propos de la détermination de la pathogénicité possible de souches avirulentes de *E. histolytica*. Dès que l'existence de *E. hartmanni* comme espèce est définitivement confirmée, je crois que le plus grand problème à résoudre est l'existence possible de souches de *E. histolytica* définitivement apathogènes, parmi celles avirulentes isolées de porteurs asymptomatiques.

Nous disposons maintenant d'une méthode éfficace pour cette différentiation, grace à Thompson et à Neal.

On injecte les souches récemment isolées dans le cœcum de jeunes rats. Les souches qui se révèlent virulentes sont des souches pathogènes en phase virulente. Les autres, qui apparaissent comme avirulentes, doivent être additionnées de bactéries isolées à partir d'une souche virulente d'amibes et doivent être passées de 2 à 15 fois

de suite dans le foie de *Cricetus auratus*. On porte ensuite les amibes en culture et on les injecte dans les cœcums d'un nouveau groupe de rats. Si elles deviennent alors virulentes, il s'agit de souches pathogènes qui se trouvaient chez l'homme dans une phase d'avirulence temporaire, mais qui peuvent toujours devenir dangereuses.

Les autres souches, qui restent toujours avirulentes, pourraient être considérées comme véritablement apathogènes.

On voit donc que les travaux de Okamoto, Rogova, Avakyan, Schensnovich et les premiers travaux de Neal jusqu'à 1956, tous travaillant directement sur le rat, sans passages hépatiques dans le hamster, ne nous indiquent pas combien de souches avirulentes isolées à partir des porteurs asymptomatiques étaient génétiquement apathogènes, et combien de ces souches étaient par contre pathogènes mais dans une phase d'avirulence temporaire.

L'expérience que nous avons de cette méthode de récupération de la virulence par passage dans le foie des hamsters nous permet d'affirmer qu'elle donne généralement de bons résultats et qu'elle pourrait être adoptée pour l'étude de la distribution des diverses souches de *E. histolytica*, pathogènes et apathogènes, dans le monde.

Dr. N. Swellengrebel (Netherlands)— Prof. Swellengrebel asks what relation exists between the following avirulent strains of *E. histolytica:*

(1) *E. dispar* of Brumpt non-virulent to kittens;

(2) Dr. Neal's avirulent strain which is, nevertheless, pathogenic to kittens.

Dr. M. M. Brooke (U.S.A.) — Although we seem to encounter a greater number of amebic diagnostic problems in the United States than disease problems, it must be pointed out that clinical cases of amebiasis do certainly occur in the temperate areas of the world. We must recall the recent outbreak in South Bend, Indiana, where at least 31 cases with 4 deaths occurred; the endemic situation in the institution for the feebleminded in Woodward, Iowa; and the hundreds of cases of amebic dysentery in the Korean prisoner of war camp. In line with Dr. Elsdon-Dew's comment, all of these occurrences involved sewage comination of water or the ready spread of fecal material from person to person.

I am sure that all of us would agree that a reliable serologic procedure would be most helpful. Unfortunately, the recently proposed Moan precipitin test has not proved satisfactory in two evaluations by a commission in the United States. Furthermore, not all of us have been as successful with the complement fixation test as Dr. Bozicevich. While in Korea, sera were collected from cases of amebic dysentery cases with demonstrated lesions on sigmoidoscopy. When they were tested at the Communicable Disease Center (Atlanta, Georgia), only about 45 % gave positive CF results. Even more disturbing, however, was the fact that, during the same period, approximately 40 % of the sera routinely received from various parts of the United States also gave positive reactions. Subsequently we have discontinued the test as a diagnostic service. The practicing physician is most anxious to use a serologic test in the diagnosis of his patients — it avoids the disagreeable problems concerned with collecting and examining of stool specimens. However, until a simple, reliable serologic procedure is developed and thoroughly evaluated, emphasis had best be placed on the direct methods.

Dr. Manson-Bahr commented that permanently stained preparations are time consuming and unnecessary. If we had enough «Manson-Bahrs» to staff all of the clinics and hospitals, I would agree. However, since we do not, it would be advisable for us to stress, particularly in the temperate areas, the confirmation of *E. histolytica* infections in permanently stained preparations. Less errors will result and consultation on difficult specimens can be obtained. The procedure need not be time consuming since rapid methods, for example Wheatley's trichrome method, can be used. The booklet *Amebiasis — Methods in Laboratory Diagnosis,* which I prepared for distribution at this time, gives the details of this and other methods.

Dr. Bozicevich (U.S.A.) — I wish to take issue to the remarks made by Dr. Brooke that the complement-fixation is not a good test. I feel that the falsely positive reactions obtained in his laboratory are due to improper control of the test. For the serum control most laboratories use the full amount of complement. This will not show the anticomplementary properties of the serum.

In our laboratory, we employ a 3 tube control test on the serum. The first tube contains 2 units of the complement, the second tube contains 3 units of the complement and the third tube the full amount of complement, or 4 units.

The following example will illustrate my point:

*The usual procedure*

| | Serum plus antigen | Serum control |
|---|---|---|
| 1:5 dilution | ++++ | — |

In the above test, he would regard the results as a positive test, since his serum control showed no fixation of complement. Let us use the same example with our procedure

| | Serum plus antigen | Serum control | | |
|---|---|---|---|---|
| | | 2 units | 3 units | 4 units |
| 1:5 | ++++ | ++++ | ++ | — |
| 1:10 | — | + | — | — |

The above example shows the value of proper serum control. Approximately 75 % of the complement is fixed by the serum itself. We would not report this serum as positive. If this serum was positive, the 1:10 serum dilution would be positive in this example.

In addition, complement deterioration which might occur is taken into consideration. Thus, 1 and 2 units of complement control are also employed.

The reference to our method is given in my presentation this morning.

2ND SESSION: **The host-parasit relationship**

*Tuesday, September 9th — 3:00 to 4:30 p.m. Room C*

The Chairman of the meeting was Dr. R. N. Chaudhuri and the Rapporteurs present were Dr. R. Elsdon-Dew and Dr. J. C. Basnuevo. The Secretary was Dr. L. T. de Almeida Franco.

The following papers were presented and discussed.

## «FULMINATING ABSCESS» ET ANATOMIE PATHOLOGIQUE

P. KIRSCHE

Institut Pasteur, Dakar

L'intention de cette communication est d'appeler l'attention sur une forme grave de réaction hépatique au cours de l'amibiase. Elle a également le dessein de proposer une pathogénie que des expérimentations ultérieures permettront peut-être de confirmer ou d'infirmer.

En Extrême-Orient, où les abcès amibiens du foie sont fréquents, il est classique de décrire des abcès francs, uniques ou multiples et de gravité moyenne; cette forme d'amibiase hépatique a été particulièrement bien étudiée avant 1945 par Huard et ses collaborateurs et élèves de la Faculté de Hanoï, dans de nombreuses communications ou thèses qui ont porté principalement sur l'aspect chirurgical, alors que le Médecin Général Blanc, après 1945, en explorait l'aspect médical, comme il vous l'exposera lui-même.

Or l'attention des chirurgiens pendant les opérations au Sud Viet-Nam a été attirée sur l'importance des cas (9 % des manifesta-

tions chirurgicales de l'amibiase) de lésion hépatique d'allure particulièrement grave. Sur 37 cas recensés, 86,5 % de mortalité, alors que les abcès francs n'en occasionnaient que 7,3 %.

Aussi en 1955 M. André évoquait la possibilité de mécanismes différents sur lesquels nous aurons à revenir plus loin.

En A. O. F., où cette affection est pourtant relativement moins fréquente qu'au Sud Viet-Nam, j'ai eu l'occasion d'examiner les pièces d'autopsie de deux très jeunes enfants africains de 16 et 24 mois dont l'amibiase a évolué en une quinzaine de jours avec les lésions hépatiques qui seront rapportées plus loin; celles-ci diffèrent très sensiblement des abcès francs amibiens classiquement décrits et se rapprochent de celles observées au Sud Viet-Nam.

## FORME CLASSIQUE

### *Clinique.*

La clinique nous enseigne que l'évolution est en général lente et progressive; elle aboutit à une suppuration bien collectée, qui se traduira par des abcès soit uniques, soit multiples.

Le malade se présente comme un grand infecté.

Le laboratoire permet de constater une polynucléose importante et une vitesse de sédimentation accélérée, point auquel le Médecin Général Blanc attache à juste titre une très grande valeur. Il permet en outre de confirmer une amibiase colique en général connue du malade et de son médecin.

L'évolution en est de façon normale favorable et une thérapeutique médicale ou médico-chirurgicale permet d'obtenir une guérison plus ou moins rapide, mais généralement définitive, dans la proportion de 85 % à 90 % des cas.

### *Anatomie pathologique.*

Les caractéristiques anatomopathologiques de cette forme à la période d'état sont les suivantes:

1° Existence d'une paroi fibreuse bien développée (*fibrous limiting wall* des Anglo-Saxons) qui sépare l'abcès du tissu hépatique

voisin (*shaggy appearance* du Dr. Elsdon-Dew). Cette paroi est le plus souvent infiltrée par des plasmocytes, des polynucléaires et des macrophages riches en pigment ocre.

Cette coque anatomiquement ne diffère guère de celle des abcès banaux.

2° Cette coque fibreuse englobe du pus qui peut avoir une coloration variable.

Dans ce pus ou dans la paroi on peut mettre en évidence des amibes et des germes microbiens.

Les statistiques de la mise en évidence des amibes sont discordantes; si Rogers, Futcher, Lacaze trouvent plus de 90 % de cas positifs, Oschner et Debakey n'en trouvent que 40 %; d'autres, et en particulier Huard et Meyer-May, 10 % environ. Ces amibes peuvent être cultivées et ont donc conservé leurs activités vitales et contaminantes. Elles auraient, par contre, perdu leur pouvoir d'enkystement.

Par contre, les auteurs s'accordent pour ne trouver guère plus de 15 % à 20 % de cas où le pus contient des germes. En général on trouve staphylocoques, streptocoques, *coli* seuls ou associés.

## FORME SURAIGUË

Cette forme classique représente, avons-nous dit, plus de 90 % des cas. On observe, dans les autres cas, des lésions hépatiques très différentes qui accompagnent des symptômes cliniques beaucoup plus graves et bien plus souvent mortels. C'est cette forme évolutive aiguë et même suraiguë qui a été assez fréquemment observée en Extrême-Orient par les Drs. M. André et P. Destombes et qui rejoint le syndrome décrit par Rogers aux Indes sous le nom de *fulminating abscess.*

### *Clinique.*

Cliniquement il s'agit de sujets dont l'état général est apparemment bien conservé. Cette conservation de l'état général contraste avec une impression d'anergie, d'anxiété, comme si le malade avait conscience de la gravité de son affection. La tension est sensiblement normale et la vitesse de sédimentation peut rester paradoxalement basse.

Il existe des douleurs abdominales violentes. Le foie est très volumineux.

Ce qui frappe dans ce tableau clinique c'est l'intensité des phénomènes généraux et en particulier l'angoisse des malades en opposition avec un état général apparemment bon, aspect habituellement observé dans les intoxications.

Le laboratoire va permettre de confirmer dans 75 % des cas, environ, l'amibiase colique le plus souvent ignorée du malade et l'absence de tout germe dans le sang et les urines.

L'évolution très rapide, en moins de 15 jours, est généralement mortelle, la thérapeutique appliquée, quelle qu'elle soit, est pratiquement impuissante, comme si dès le début de la maladie les lésions étaient irreversibles.

C'est donc le plus souvent des pièces d'autopsie qui sont adressées à l'anatomo-pathologiste et celui-ci est surpris de constater que seul le foie présente des lésions importantes, en dehors des lésions classiques observées au niveau du cadre colique et des lésions de la période agonique.

*Anatomie pathologique.*

Les lésions de cette forme vont se présenter d'une façon totalement différente de celles que nous avons pu décrire dans la forme collectée.

Ici, en effet, plus de coque conjonctive, donc pas de tentative de limitation défensive de la lésion par l'organisme. La lésion se trouve directement en contact avec le tissu hépatique apparemment sain dont les capillaires viennent s'ouvrir à plein canal dans la lésion, qui est limitée aux espaces centro-lobulaires. À la phase initiale ou à un stade plus jeune, il ne s'agit plus d'un abcès, mais d'un nodule à contours irréguliers, où les éléments semblent momifiés; leur protoplasme est oxyphile, leur noyau en pycnose. À la périphérie, les capillaires sont ectasiés, mais optiquement vides, donnant au petit grossissement un aspect feuilleté à la place de l'aspect dense du tissu hépatique normal.

Il n'existe pas de lésions du type inflammatoire. À la période plus avancée, il s'agit d'une véritable nécrose oxyphile centro-lobu-

laire ne laissant parfois qu'une seule rangée d'hépatocytes en bordure des espaces de Kiernan.

Ces escarres sont en général petites, formant des micronodules limités à l'espace centro-lobulaire, mais elles sont très nombreuses, n'étant séparées que par les quelques cellules persistant au pourtour des espaces portes. Il faut insister sur l'importance des *lésions vasculaires,* surtout évidentes au niveau des vaisseaux portes; ceux-ci sont collabés, le plus souvent même obstrués par des phénomènes de vascularite, du type nécrose fibrinoïde.

Ces lésions hépatiques sont concomittantes avec des lésions rénales et pulmonaires, mais qui sont mineures par rapport à celles observées dans le foie. Dans ces deux organes, ce sont principalement des lésions vasculaires qui sont notées également, lésions glomérulaires dans le rein, lésions des vaisseaux des parois alvéolaires du poumon. Elles diffèrent des lésions agoniques ou post-mortem.

Le laboratoire ne mettra jamais en évidence ni amibes ni germes.

En conclusion, les lésions hépatiques dans cette forme grave se traduisent par des micro-nécroses pour lesquelles aucune étiologie parasitaire ou bactérienne ne peut être mise en évidence et qui s'accompagnent de lésions vasculaires très importantes.

L'observation de ces lésions avait suggéré à André l'idée qu'elles étaient dues à des troubles vasculaires, du fait, en particulier, des nécroses multiples *patchy*.

## ESSAI PATHOGÉNIQUE

Ces lésions se rapprochent en effet énormément de celles étudiées en 1951 à la dixième conférence américaine sur les lésions hépatiques. L'article du Dr. Rappaport, lu par le Dr. Best et à la discussion duquel prirent part les Drs. Artroft, Fremont, Smith, Gyorgi, etc., démontrait qu'une nécrose centro-lobulaire «embracing wave of widespread central necrosis» avec une persistance du parenchyme périportal est caractéristique de l'ischémie expérimentale, obtenue chez le chien par des ligatures progressives des artères hépatiques, associées à une fistule d'Eck. La mortalité au cours de cette expérimentation est très élevée.

Il a paru logique de penser, par analogie, que l'obstruction pathologique de ces mêmes vaisseaux puisse provoquer des lésions identiques.

L'expérience de Rossle et de Frolich sur le mésentère de grenouille a démontré que chez l'animal sensibilisé, au lieu d'application de l'antigène, on observait une dilatation des vaisseaux, suivie d'un dépôt de fibrine dans la lumière de ces vaisseaux, d'où thrombose et, à la periphérie, une zone de dilatation des capillaires qui, eux, sont vides d'hématies. Il semble prouvé qu'il s'agit d'une stase qui provoque l'œdème. Cet ensemble a été désigné sous le nom de *vacuole plasmatique*. Les lésions vasculaires sont du type nécrose fibrinoïde.

Nous avons retrouvé ces différents aspects sur les lames examinées. Il faut essayer d'en déterminer l'étiologie.

Il est habituellement admis que l'amibe ne peut se développer sans l'existence d'un complexe amibe-bacille que M. André a dénommé *souche amibienne*.

Dans l'amibiase suraiguë les lésions intestinales coliques provoquées par cette association sont portées à leur maximum sans qu'il soit possible de faire la part de ce qui revient à l'amibe et de ce qui revient aux germes microbiens. Il existe une libération de substances protéiques par destruction cellulaire et cette libération a une répercussion sur la constitution du sérum sanguin; les travaux indiens (Heilig et Visveswar Chitkara et Chuttani), anglais (Shute) et français (Charmot) montrent que la sérum albumine peut être diminuée et la sérum globuline augmentée. J'ai pu, dans un cas grave observé à Dakar, constater une nette augmentation de la sérum globuline.

Il devient alors logique de rapprocher ces constatations des travaux récents sur les lésions par introduction des $\gamma$-globulines dans l'organisme; il était possible de penser que l'obstruction de ces mêmes vaisseaux pourrait être provoquée par une coagulation intravasculaire consécutive à la libération de substances thromboplastiques que l'on peut retrouver, par exemple, dans les cancers à fort pouvoir nécrotique. On observe des lésions du même type dans les accidents vasculaires obstétricaux, comme l'a rappelé le Prof. Agrégé de Brux. Il s'agit en réalité de phénomènes plus ou moins brutaux parfois à allure de shock. C'est ici le lieu de rappeler le travail initial de Klinge et Vaubel en 1930, qui fut à l'origine des recherches de Hawn et Janeway, les Cavalti, Murphy et Swift, Moore et coll., Wissler et coll., et, plus récemment, ceux du Prof. Agrégé de Brux et de ses collaborateurs. Il en résulte que les injections d'albumine sérique peuvent provoquer des shocks aigus et que les $\alpha$-globulines occasionnent

des lésions plus brutales et plus accusées que les γ-globulines. Il existerait dans le sérum de certains malades, et dans certaines conditions, un facteur nécrosant qui serait fixé dans la fraction II de Cohn et qui migrerait avec les globulines. C'est l'hypothèse soutenue par Godman et ses coll. et par Gueft et qui expliquerait, par exemple, l'existence du facteur de Hasserick.

Les lésions des hépatites amibiennes suraiguës pourraient donc être dues à des lésions vasculaires, elles-mêmes provoquées par des lésions de shock du type Sanarelli Schwartzmann. Il peut, certes, exister des causes adjuvantes, et l'hypothèse de M. André concernant le rôle du traitement par l'iode ne peut être écartée. Ce traitement donne le plus souvent des résultats très intéressants, comme l'a souligné le Médecin Général Blanc, et il ne semble pas nécessaire de faire intervenir ces complexes iodoprotéiques pour expliquer ces lésions.

En résumé, la majorité des amibiases hépatiques obéissent aux critères classiques et se traduisent par des abcès francs, avec ou sans amibes et bactéries, et leur aboutissement normal est la guérison avec ou sans séquelles.

Il reste environ 9 % des cas où l'on a pu constater que ces critères n'étaient plus valables. La nature amibienne des lésions observées ne peut jamais être mise en évidence, les lésions nécrotiques restent localisées au début à la région centro-lobulaire et cependant la mortalité est très élevée. Dans ces conditions il semble permis de rechercher un autre processus étiologique. On est fondé à incriminer les produits de désintégration de la paroi intestinale ou l'exacerbation des produits toxiques élaborés par les bactéries d'accompagnement, peut-être la toxicité de certains produits thérapeutiques ou l'association de ces diverses étiologies, le tout étant susceptible d'engendrer une augmentation des γ-globulines circulantes et les lésions qui en découlent, selon certains auteurs.

Cette hypothèse soulevée par M. André et par J. Reilly, à la suite des observations de cas de cette nature en Extrême Orient, devrait pouvoir donner lieu à des confirmations expérimentales et à des essais thérapeutiques antistress.

Fig. 1 — Nodule nécrotique

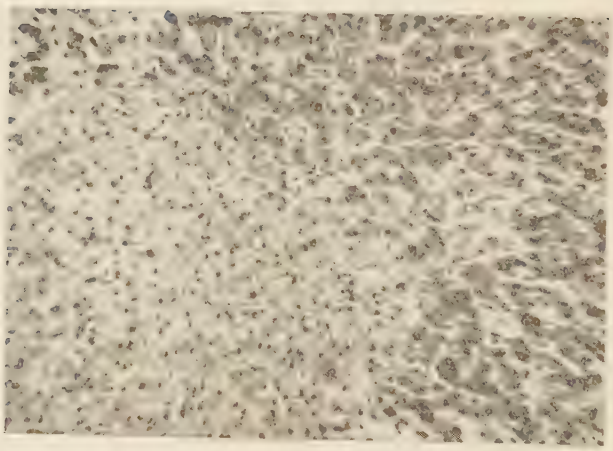

Fig. 2 — Bord mal limité du nodule

Fig. 3 — Zone perinéale sans coque

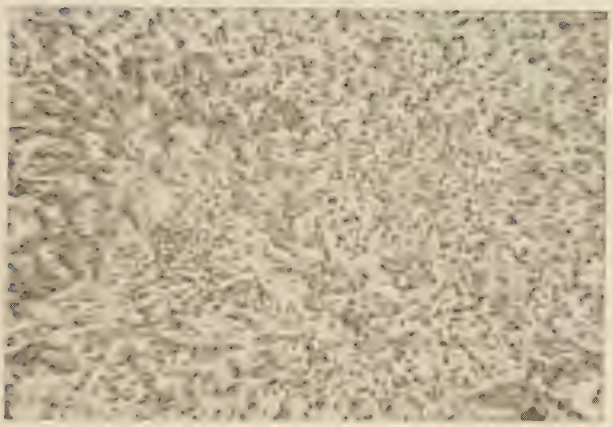

Fig. 4 — Infiltration : aspect feuilleté

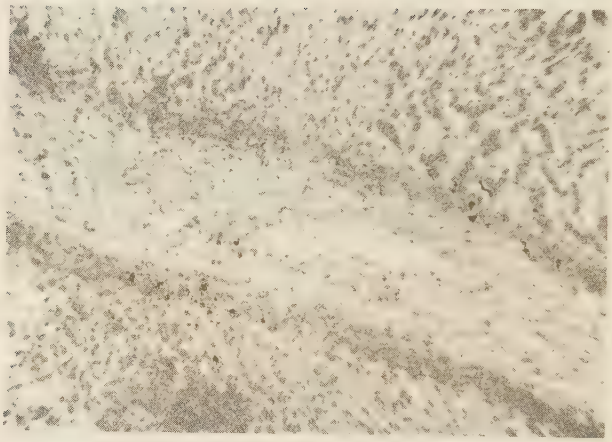

Fig. 5 — Thrombose vasculaire

# THE PATHOLOGY OF AMOEBIASIS

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Durban, a city of approximately half a million, is peopled by equal numbers of Europeans, Africans and Asiatics. The picture of amoebiasis presented in this paper is drawn from necropsy or biopsy material mainly from King Edward VIII Hospital, which admits Africans and Asiatics only. Between 2 and 3 thousand cases of amoebiasis are seen annually, of which the vast majority are Africans. The majority of these present as an acute amoebic dysentery which responds rapidly and satisfactorily to treatment, but among the Africans an acute fulminating disease is sometimes seen and it is from this group that much of our necropsy material has been obtained. Of 230 post-mortems on cases where death was due to, or associated with, amoebiasis in the period 1951 to 1955, 62 % were attributable to acute fulminating dysentery. In 20 % of these acute dysenteric cases liver abscess was also found. It is not proposed to deal with the well-known type of amoebic dysentery, but rather to limit the discussion on dysentery to the acute fulminating type. In addition, the bowel complications, and liver abscess and its complications will be discussed.

## ACUTE FULMINATING AMOEBIC DYSENTERY

A number of these patients are moribund on admission. The usual history of diarrhoea with blood and mucus, abdominal pain and tenesmus is short, with an average duration of two weeks. At necropsy

there is almost invariable a generalised peritonitis. In only a minority of these cases does actual perforation occur. There appears to be a seepage especially from areas of ulceration which are visible from the peritoneal surface. The colon is grossly dilated and extremely friable; it is usually impossible to remove this organ intact. The bowel wall averages 1 cm in thickness. On opening the colon almost the whole mucosa and sub-mucosa is replaced by a soggy slough.

Only isolated strips or islets of apparently viable mucosa remain. Occasionally the terminal 10 cm of ileum is involved as is the perianal skin. Cases surviving a few days may show separation of the slough, the bowel then being lined by ragged muscularis interna and sub-peritoneal tissue. Histology of the colon in these cases shows necrosis extending into and often through the main muscle layers. Amoebae are seen not only at the junction of necrotic and viable tissues, but deeper in the tissue spaces and in small vessels.

## COMPLICATIONS OF AMOEBIC DYSENTERY

*Localised peritonitis.*

This occurs principally where the dysentery is more localised and is due to perforation. Perforations of the descending and ascending colon are often retroperitoneal or localised in the paracolic gutters. Pericolic abscess may result and these abscesses occasionally tract down into the inguinal regions. In one case at necropsy the psoas sheath was penetrated with the result that a psoas abscess pointed in the thigh.

*Amoebic appendicitis.*

The appendix is involved in only 10 % to 20 % of cases. The whole mucosa is affected, together with the adjacent caecum. It is not surprising that in the event of appendicectomy great difficulty is experienced with closure of the stump.

*Intussusception.*

Amoebiasis is a common cause of intussusception in adult patients in King Edward VIII Hospital. Of the cases seen some have developed during anti-amoebic treatment. The apex of the intussusception is usually at the ileo-caecal junction.

One case was seen at necropsy where the intussusception commenced in the transverse colon. Increased peristalsis and local oedema are the probable causes of intussusception.

*Intramural amoeboma.*

We regard this form of amoeboma as a sequel to a slow perforation, the main complaint being one of colonic obstruction. Biopsy specimens from the transverse colon show abscess formation in the muscle extending into the omentum, which is adherent over the site of perforation. There is surrounding fibrosis and this, together with the abscess, causes marked narrowing of the bowel lumen. The granulomatous tumours projecting into the bowel lumen, seen clinically in the rectum and pelvic colon, have not been observed in any of our necropsy cases.

*Ischiorectal abscess.*

Two biopsy specimens have been seen and in both cases necrosis extended to the adipose tissue. Many amoebae could be seen in the sections. Wherever amoebiasis is prevalent, its possible association with ischiorectal abscess should be remembered.

*Sigmoido-vesical fistula.*

Only one case of this extremely rare complication has been seen at necropsy. Large sloughing ulcers were present in the caecum, transverse colon and the sigmoid colon. The sigmoid was adherent to the bladder and a large fistula was present between the two organs. This patient had in addition eight liver abscesses, each 3-4 cm in diameter.

*Colonic stricture.*

No case of this complication was encountered in our necropsy series. The only specimen seen was removed surgically following perforation of a stercoral ulcer proximal to an obstruction. There was marked fibrosis of the stricture and evidence of healing of deep ulceration.

*Anal amoebic granuloma.*

Biopsy material has been seen in a number of these cases, often in the absence of dysentery. Some present an anal condyloma accuminata while others have clinically suggested an anal carcinoma. Histologically, there is epidermal hyperplasia with focal areas of ulceration, in which many haematophagous amoebae can be seen.

*Invasion of the vagina and cervix.*

This complication has been seen in a few necropsy cases with severe dysentery in whom recent abortion or full-term delivery has occurred. Two cases of cervical amoebiasis have recently been encountered from biopsy specimens. The one case presented as an erosion and was biopsied following failure of the usual treatment. Amoebae were found although there had been no evidence of colonic amoebiasis. The second case was investigated after her husband had been found on biopsy to have an amoebic lesion of the penis. At examination a small cervical erosion was found and numerous amoebae were present in the preparation of the fluid. A further case of penile amoebiasis has recently been seen. This had presented as a phagedena. Circumcision was performed and numerous amoebae were present in the prepuce.

## LIVER ABSCESS

In this series of 230 necropsies on cases of amoebiasis, liver abscess was found in 77 cases, of which 54 were single and 23 multiple. The great majority of abscesses occurred in the right lobe of the liver. In over 90 % of liver abscesses at necropsy the abscess contents

were yellow. Only rarely does the liver abscess contain the «anchovy-type» material considered by many to be characteristic.

The earliest macroscopic lesions appear as minute yellow foci similar to that in fatty change. These necrotic areas are multifocal, but, with coalescence, generally a single large abscess results. Histology shows necrosis of parenchymal cells and amoebae within the sinusoids. Initially the necrosis is zonal and centrilobular with a few surviving parenchymal cells around the portal tracts. In early abscesses the portal tracts are preserved and reticulin stains demonstrate that the liver framework is intact. With extension of this process, large solid areas of necrosis become manifest with appearances suggestive of caseation. Even abscesses of 8 cm in diameter may show only focal areas of liquefaction. Histology reveals a coagulative necrosis with amoebae most numerous at the spreading edge of the abscess. Inflammatory cell infiltration is relatively insignificant and initially confined to the portal tracts. Inflammatory cells are mainly lymphocytes, plasma cells and macrophages and appear to be directed at the necrotic tissue rather than at the amoebae.

In the active amoebic abscess there is no apparent attempt on the part of the host at limitation or walling off of the necrotic process. The absence of fibrosis around all but chronic abscesses is well illustrated by one of our necropsy cases. This patient received emetine and had had his liver abscess aspirated with a yield of 500 ml of pus. The patient was discharged cured. He was re-admited some 8 weeks later, dying shortly after admission, from an unrelated lobar pneumonia. At necropsy, the only evidence of the previous abscess was a scar 1 cm in diameter in the liver.

## COMPLICATIONS OF LIVER ABSCESS

*Rupture into the pericardium.*

Eight cases of rupture of left lobe abscesses into the pericardial cavity were encountered in this necropsy series. These cases had a chronic type of amoebic liver abscess with a fibrous connective tissue wall. All the abscesses were adherent to the diaphragm at the attachment of the pericardium. Most of the cases seen died within a

day or two of the rupture. In one case the condition was of longer duration and commencing organisation in the pericardium was present. Should these patients survive without adequate drainage and anti-amoebic treatment, it appears possible that a constrictive pericarditis could result. Histologically the subpericardial myocardium shows oedema and some inflammatory cell infiltration. No amoebae were found in the sections of pericardium of these cases, though they were still present in the wall of the liver abscess in approximately half the cases.

*Rupture into the right lung and pleura.*

Only five cases of rupture of right lobe abscess into the lung or pleura were encountered in this necropsy series. The lower frequency of this complication by comparison by rupture into the pericardium is due to the fact that rupture into the lung is less acute and, being recognised clinically, is often efectively treated. An abscess with relatively acute perforation through the diaphragm ruptures into the pleural cavity, while those in which diaphragmatic pleurisy has had time to occur rupture into the lung substance. One right lobe abscess was encountered which had ruptured both into the lung and into the lumen of the colon. Segments of *Taenia saginata* were present in both lung and liver abscesses. No cases of amoebic abscess of the lung in the absence of liver abscess have been encountered in this series.

*Rupture into the peritoneal cavity.*

Though this complication is not infrequently encountered clinically, only one case came to necropsy.

*Jaundice.*

Two cases, where jaundice occurred in the presence of liver abscess, came to necropsy. The one case, an adult, showed a mild obstructive type of jaundice associated with a 10 cm abscess in the right lobe which caused pressure on the hepatic ducts. The other case was that of a 4-month old infant who died shortly after admission. The history was one of deepening jaundice over the previous 4 weeks.

At necropsy, two large abscesses were present wich, by pressure and distortion of the biliary system, produced an obstructive jaundice and a mucocoele of the gall bladder.

*Portal obstruction.*

Two cases have been seen at necropsy. The first was an adult who presented with ascites and splenomegaly. At necropsy he was found to have a chronic right lobe abscess, causing pressure on the portal vein. The second case was that of an infant with a large abscess enroaching on the portal fissure with resultant portal vein thrombosis.

*Brain abscess.*

In this series of necropsies, two cases of amoebic brain abscess were seen. Both were associated with liver abscess.

*Amoebic hepatitis.*

In the routine histological examination of the liver of all our necropsy cases, we have not seen a single case of diffuse hepatitis attributable to the amoeba. In some cases presuppurative lesions are present in association with liver abscess. In one case of amoebic dysentery, focal liver necroses, resembling those of typhoid fever, were seen, but no amoebae were found in the sections of liver. From neither necropsy nor biopsy material have we been able to find «diffuse non-suppurative amoebic hepatitis».

## CONCLUSIONS

From our experience in a large series of observations, it is apparent that amoebiasis is frank in its pathological manifestations and there is no evidence of lesions remote from the amoebae themselves. Amoebae can cause extensive lesions and in endemic areas are an important cause of morbidity and mortality.

TABLE 1

| *Age distribution* | | % |
|---|---|---|
| Under 1 year | 6 | 2.6 |
| 1–2 years | 20 | 8.7 |
| 3–5 years | 10 | 4.3 |
| 6–10 years | 5 | 2.2 |
| 11–20 years | 3 | 1.3 |
| 21–30 years | 40 | 17 4 |
| 31–40 years | 55 | 22.9 |
| 41–50 years | 40 | 17.4 |
| 51–60 years | 29 | 12.6 |
| 61 and over | 22 | 9.6 |

Of patients 20 years and over 71 % are males.

TABLE 2 — *Specific cause of death*

| | | | % |
|---|---|---|---|
| *Intestinal:* | | | |
| Fulminant dysentery | Without peritonitis | 21 | 9.1 |
| | With peritonitis and perforation | 12 | 5.2 |
| | With peritonitis and without perforation | 110 | 47.8 |
| Localised dysentery with perforation | | 8 | 3.5 |
| Pericolic abscess | | 4 | 1.7 |
| Intussusception | | 3 | 1.3 |
| Ischiorectal abscess | | 1 | 0.4 |
| *Liver abscess:* | | | |
| Without rupture | | 33 | 14.4 |
| Rupture into | Pericardium | 8 | 3.5 |
| | Lung or pleura | 5 | 2.2 |
| | Peritoneum | 1 | 0.4 |
| *Brain abscess* | | 2 | 0.9 |
| *Coincident pathology* | | 22 | 9.6 |

ACKNOWLEDGMENTS

I have to thank Dr. R. Elsdon-Dew and Dr. J. Wainwright for help and encouragement, and Dr. S. Disler, Superintendent of King Edward VIII Hospital, and Dr. H. D. Tonking, Provincial Pathologist, for permission to publish.

# THE EXUDATES IN AMEBIC COLITIS [1]

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Early studies on the differential microscopic characteristics of the bowel discharges in amebic and bacillary dysenteries revealed a number of distinctive features of each (2, 5, 6, 7, 14, 15, 22, 26). The comparative cytology of the exudates in various other types of dysentery also has been described (3, 24). In amebic dysentery the stool generally has been characterized as having relatively scanty cellular elements, an abundance of «pyknotic bodies» and, usually, Charcot-Leyden crystals. Only Anderson (2) was impressed by the presence of eosinophils. He stated that «the prevalence of eosinophils is so noticeable that it might almost be regarded as a pathognomonic sign of the presence of entamoebae». It was also Anderson who first remarked on the agglutination of red blood-cells in the stools. This observation on «Anderson's phenomenon» has been confirmed by almost all workers. Although his observation on the conspicuous presence of eosinophils has not been confirmed, some writers have accepted that feature as characteristic of amebic dysentery (1).

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[1] Contribution from The Amoebiasis Research Unit, University of Natal Medical School, Durban, South Africa, and Department of Tropical Medicine and Public Health, Tulane Medical School, New Orleans, Louisiana, U.S.A.

[2] I wish to thank my colleagues Dr. Ronald Elsdon-Dew, Director of The Amoebiasis Research Unit, and Drs. A. J. Wilmot and S. J. Powell, of the Department of Medicine, University of Natal, for direct assistance with the planning and carrying out of this study; and to acknowledge the support and assistance of the South African Council for Scientific and Industrial Research.

If Charcot-Leyden crystals are derived from eosinophils, as is commonly held, when they are present in dysenteric stools, eosinophils should generally accompany them [1]. It cannot follow that the presence of either is a certain indication of amebic infection, however, since it is known that they are characteristic elements of the exudates in other forms of colitis. It is particularly to be noted that both eosinophils and Charcot-Leyden crystals are abundantly present in cases of diarrhea and dysentery resulting from heavy *Trichuris trichiura* infections. *Trichuris* and amebic infections cause much the same kind of disease, including grossly indistinguishable stools. In the experience of some workers, when the two infections occur together, as they often do, removal of the worms results in prompt relief of symptoms whereas elimination of the amebae does not (13, 19, 20). Detailed observations on the bowel exudates in *Trichuris* dysentery have not been reported. Generally, the identification of this type of dysentery can be based on the number of eggs in the stool and the presence of visible worms attached to the rectal mucosa.

Workers whose interests include either amebic or *Trichuria* dysentery have followed with keen attention a series of studies by Elsdon-Dew and his associates at the Amoebiasis Research Unit in Durban, South Africa (8, 9, 10). Among urbanized native Africans, but not others, in Durban, amebic dysentery is peculiarly common and extremely severe. *Trichuris* dysentery, not uncommon in other parts of the world, has not previously been studied in the African at Durban. However, *Trichuris* infection, present in only 30 % of newcomers from outlying districts, is found in approximately 60 % of those who have lived a year or more in Durban, where amebic infections, earlier apparently well tolerated if present, tend to produce fulminating dysentery (11). These circumstances suggest a possible relationship between the type of amebic dysentery seen in the African at Durban and colon-damaging helminthic infections that may be associated with it.

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[1] It is doubtful that Charcot-Leyden crystals may occur in either dysenteric or other types of stools without some degree of eosinophilia of the intestinal tract. They are soluble in hydrochloric acid at pH 2.4 and are therefore unlikely to occur in the feces except when they enter the bowel stream below the stomach (18).

The present study was undertaken to determine whether the microscopic characteristics of the bowel exudates could be made use of in assessing the importance, if any, of concomitant helminthic infections either as predisposing agents or complicating factors in amebic dysentery as seen in the African at Durban.

## MATERIALS AND METHODS

Observations were made on the stools and proctoscopic scrapings from 35 adult male Africans, 21 to 51 years of age, admitted to King Edward VIII Hospital in Durban for treatment of amebic dysentery. All except one were among a series of patients used in drug-trial studies by Elsdon-Dew and associates whose routine methods have been described (29). On the basis of physical and proctoscopic examinations, the patients were managed in one of three ways. There were 20 who where given trial treatments using new antiamebic drugs. Proctoscopic examinations were made 5, 10, 15, 20 and 27 days after the beginning of treatment and stools were examined at frequent intervals. There were 12 with severe ulceration requiring immediate reliable treatment. At the end of treatment, usually about 10 days, they were again given proctoscopic examination and, if sufficiently improved, were transferred for follow-up. There were 3 who were immediately transferred for simultaneous treatment of other ailments. More than half (21) of the group had histories of previous dysentery, mostly with a diagnosis of *E. histolytica*. The periods of complaint in the present illness varied from 2 days to 1 year; 17 had been passing bloody stools for 2 weeks or less, 9 for 1 week or less. At the beginning of treatment 8 of the group had some degree of fever which persisted less than 48 hours in 6, and less than 5 days in the others. Stool cultures for bacterial pathogens revealed *Salmonella* in one individual who was afebrile and had relatively scanty pus in the stool.

As a routine, examination was made of at least one fresh stool before the initial proctoscopy, at which time scrapings from ulcers also were obtained for study of the exudates. From 18 individuals slice biopsies for histopathological study and examination for eggs of *Schistosoma mansoni* were obtained. At subsequent proctoscopic exam-

inations additional scrapings were taken if ulcers were present. In most cases dysentery and diarrhea were checked within 1 to 5 days, but in approximately half of the cases ulceration was present 10 days or more after the beginning of treatment. In 6 instances after the first treatment had failed, a second was given using a drug of proven effect.

Stools were examined for parasites in saline smears, and by zinc sulfate flotation or formalin-ether sedimentation, or both. Direct smear counts (eggs per 1-2 mg feces) were made of *Trichuris*, hookworm and *Ascaris* eggs. Exudates were examined in temporary mounts using Randolph's (25) stain and in permanent mounts after fixation in Schaudinn's or Bouin's solutions and stained with Giemsa, or fixed in Schaudinn's solution and stained with iron-hematoxylin. In wet-fixed, Giemsa-stained smears all cellular elements were identifiable. The amebae were well preserved and well stained, and after Schaudinn's fixation, Charcot-Leyden crystals were well preserved. Iron-hematoxylin stained the Charcot-Leyden crystals somewhat better, but amebae were as easily detected and identified in Giemsa-stained smears. Giant epithelial cells, apparently the same as those described by Boddington and Truelove (4) in proctoscopic scrapings from individuals with ulcerative colitis, were conspicuous in Giemsa- but not in hematoxylin-stained smears. Estimates of relative abundance or prevalence of cells and Charcot-Leyden crystals, based on examination of entire smears made from a number of different samples when available, were recorded as four classes: lacking or very scanty (±); readily found but not numerous (+); numerous and generally distributed or abundant in scattered areas (++); and abundant in numerous areas (as in Fig. 1) or massive in scattered areas (+++).

## OBSERVATIONS

Eosinophils, neutrophils, Charcot-Leyden crystals, giant epithelial cells and sloughed mucosal cells, including patches of epithelium, in addition to blood, mucus and parasites were the only conspicuous elements recognized. Lymphocytes, plasma cells, other round cells and occasionally macrophages were present, but they were too few to contribute significantly to the character of the exudates. Sloughed

tissue cells in various stages of degeneration up to «pyknotic bodies» were invariably present in distinctly dysenteric stools. In about one-third of the stools examined before treatment patches of mucosal epithelium were observed, and in three instances entire mucosal glands were found free in the stools. Clumping of red blood-cells was occasionally very pronounced, but in general it was not a conspicuous feature. It was unquestionably present in 15 of the 35 individuals, markedly so in 10 (Table 1).

TABLE 1 — *Frequency and relative abundance of eosinophils, neutrophils, giant epithelial cells and Charcot-Leyden crystals in 1 to 6 stools or proctoscopic scrapings from 35 individuals with amebic dysentery, examined before, during and after treatment*

| | +++ | ++ | + | ± |
|---|---|---|---|---|
| Eosinophils | 25 | 7 | 2 | 1 |
| Neutrophils | 4 | 12 | 15 | 4 |
| Giant epithelial cells | 5 | 10 | 16 | 4 |
| Charcot-Leyden crystals | 9 | 6 | 7 | 13 |

*Gross characteristics of stools.*

Before treatment all stools contained large quantities of mucus and at least some blood; many contained no feces. In 5 individuals, all with mild fever, mucus in the stools was more or less uniformly cloudy. Odor of pus was detected in pretreatment stools of 6 individuals whereas distinct proteolytic odor without pus was detectable in 20. In general, stools with detectable odor of pus contained abundant neutrophils and relatively few eosinophils, while in those with strong proteolytic odor the reverse was found.

*Microscopic characteristics of exudates.*

Before or at the beginning of treatment, eosinophilia or the bowel exudates was high in 25 and moderate in 7 individuals (Table 1). Neutrophils were very abundant in 4, moderately numerous in 12; they were not remarkable in the remainder. Giant epithelial cells were

abundant in 5, numerous in 10 and present in small numbers in 16. In 25 instances from 21 individuals giant epithelial cells were found free in the stools, plentifully in 12 of them. All stages of giant epithelial cells were seen ranging from the complete cell, differing from the normal goblet cell only in size, up to the necrotic body which in its terminal phases of degeneration became a mucoid mass occasionally incorporating smaller cells and other objects. When stained with Giemsa, it usually was a conspicuous, ovoid, basophilic body, spongy in appearance, and often seen in groups of various sizes up to about 30 $\mu$ by 60 $\mu$ (Fig. 2). Its length exceeded 70 $\mu$ in some instances, but usually it was less than 50 $\mu$. The pyknotic nucleus occasionally was dislodged from the main body, but more often it was flattened against the outer surface or situated somewhat within the cell. In fresh smears these cells sometimes were seen in large numbers and had somewhat the general appearance of amebae. Charcot-Leyden crystals were found in stools or proctoscopic scrapings from 22 individuals; they were abundant or numerous in 15. Charcot-Leyden crystals and giant epithelial cells usually were not found in the absence of pronounced eosinophilia. In 11 cases of extreme eosinophilia in which Charcot-Leyden crystals were lacking before treatment, they were found subsequently in 6 (Table 1).

Early in the treatment period, even when ulceration was still evident, neutrophils rapidly became inconspicuous in the exudates (Table 2). They were found in considerable numbers in one of 23 individuals examined at 10 days and in one of 10 examined at 15 days. On the other hand, eosinophils, giant epithelial cells and Charcot-Leyden crystals persisted in some cases up to 27 days (Table 2). It could not always be accurately determined whether in those instances amebae were still present. Among the 11 cases followed for 27 days and 6 others followed for 20 days, there were 2 in which eosinophilia of the exudates was high despite the more or less certain absence of amebae. In one of these there was a heavy *Trichuris* infection; in the other, advanced larval stages of *Strongyloides* were repeatedly found in fresh stools and proctoscopic scrapings. On the other hand, there was no instance among the cases studied for 10 days or longer in which eosinophils were not conspicuous when amebae and cellular exudates of any kind occurred together or when exudates persisted in the stools for short periods after amebae were removed

by effective treatment. In 6 instances amebae were found throughout the 20- or 27-day periods of observation. In one of them no cells could be found in the last specimen examined, but earlier ones contained moderate to high numbers of eosinophils, and a stool without

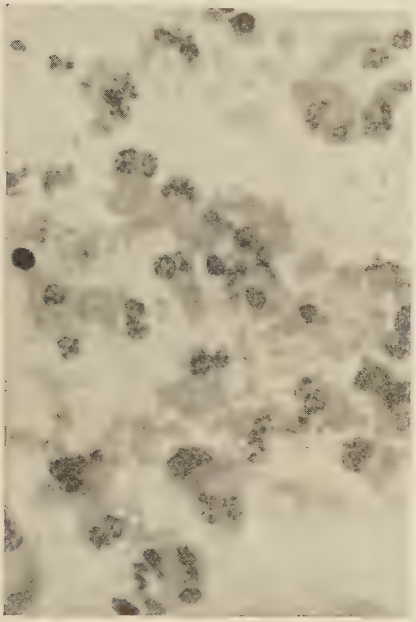

Fig. 1 — Eosinophils, some degenerate and disrupted, in Giemsa-stained smear of stool from an untreated patient with amebic dysentery

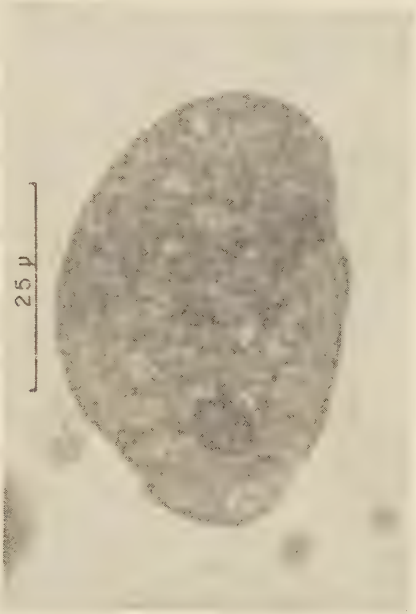


Fig. 2 — «Giant epithelial cell» (Boddington and Truelove), Giemsa-stained in stool of an untreated patient with amebic dysentery, showing remnant of the nucleus and the large, spongy body of the cell. The nucleus is often attached at the margin and may be lacking

cells 5 days before discharge contained Charcot-Leyden crystals. Among the remaining 5 in which amebae persisted throughout, the last stools contained abundant mucus laden with eosinophils in 3, while in the others there were numerous eosinophils in scanty strands of mucus scattered throughout soft stools (Table 2). In all cases where amebae persisted, only the *minuta* forms of Hoare (tropho-

TABLE 2 — *Relative abundance of eosinophils, giant epithelial cells and Charcot-Leyden crystals in bowel discharges from 35 individuals with amebic dysentery, observed before and for 5 to 27 days after the beginning of treatment*

| Days | Number of examinations [1] | | | | Neutrophils | | | Eosinophils | | | C-L crystals | | | G E cells [2] | | | Amebae present |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P&S | P | S | Total | +++ | ++ | + | +++ | ++ | + | +++ | ++ | + | +++ | ++ | + | |
| 0 | 33 * | 1 | 1 | 35 | 4 | 11 | 14 | 20 | 8 | 6 | 6 | 4 | 4 | 4 | 7 | 8 | 33 |
| 5 | 1 | 12 | 3 | 16 | 0 | 1 | 7 | 7 | 6 | 3 | 2 | 1 | 3 | 0 | 0 | 1 | 7 |
| 10 | 5 | 16 | 2 | 23 | 0 | 1 | 6 | 8 | 6 | 7 | 3 | 3 | 2 | 1 | 0 | 0 | 3 |
| 15 | 3 | 4 | 3 | 10 | 0 | 1 | 3 | 4 | 2 | 2 | 0 | 1 | 1 | 0 | 1 | 1 | 3 |
| 20 | 2 | 5 | 5 | 12 | 0 | 0 | 3 | 3 | 3 | 2 | 1 | 0 | 2 | 0 | 1 | 0 | 5 |
| 27 | 6 | 0 | 5 | 11 | 0 | 0 | 2 | 2 | 3 | 5 | 0 | 1 | 5 | 0 | 0 | 1 | 4 |

[1] Proctoscopic scrapings (P) and stool (S), or one and not the other.

[2] In stools only; giant epithelial cells observed in proctoscopic scrapings are not included.

* One individual in this group had an injection of emetine 24 hours prior to examination.

zoites and cysts) were found. This was true in one case when unhealed ulcers still were evident.

*Blood eosinophilia.*

In most cases of high eosinophilia of the bowel exudates, eosinophilia of the blood was relatively low (Table 3). Counts made on 31 individuals before treatment and on 24 at the end of observation periods were 1000/mm$^3$ or higher in 12 individuals, above 2000/mm$^3$ in 3. The highest was 4000/mm$^3$ (24 % of 17,000 WBC) (Table 3). Numerous individuals with very abundant eosinophils in the bowel exudates had less than 500 eosinophils/mm$^3$ in the blood. Correlation between blood eosinophilia and either exudate eosinophilia or helminth infections was therefore not evident. In all of the 3 individuals harboring no helminths or none likely to have any effect on the colon, eosinophil levels were low in the blood, high in the stools, and Charcot-Leyden crystals were abundant in one, scanty in another.

*Helminth infections* (Table 4).

Helminths that might influence the character of the bowel exudates were found in a majority of the 35 individuals studied. Only one was negative for all helminths. *Trichuris* was found in 32; *Ascaris* in 16; *S. mansoni* in 4; hookworm, *Strongyloides* and *Taenia* each occurred in 5. There were various combinations and 2 individuals harbored 4 different helminths (Table 4).

Damage to the colon is known to be caused by three of these helminths — *Trichuris, S. mansoni* and *Strongyloides,* the latter perhaps only when there is internal autoinfection This condition, probable in 2 of the 5 *Strongyloides* infections, was suggested by the presence of advanced-stage larvae in fresh stools and proctoscopic scrapings. The *S. mansoni* infections all appeared to be light and there was no evidence that they were responsible for symptoms. It was similarly difficult to determine the degree of damage to the colon attributable to the *Trichuris* infections. Worms were not observed at proctoscopy in any case. The range in worm burden, estimated by egg counts, was considerable and there were 8 instances in which *Trichuris* was found together with either *Strongyloides* (4), *S. mansoni* (3) or both (1).

TABLE 3 — *Selected cases with highest eosinophilia of the blood and the associated helminth infections, Charcot-Leyden crystals and eosinophilia of the bowel exudates*

| Case number | Before treatment | | After treatment | | Exudates | | Helminths |
|---|---|---|---|---|---|---|---|
| | Total WBC | % eos. | Total WBC | % eos. | Eos. | CLC | |
| 17867 | — | — | 17 | 24 | +++ | +++ | *T.t.* 124/s; *Taenia* |
| 18451 | 14 | 20 | 10 | 30 | +++ | +++ | *T.t.* 23/s; *Str.* L 1; *S.m.; As.* 16/s. |
| 19213 | — | — | 18 | 13 | ++ | − | *T.t.* 2/s; *As.* 176/s. |
| 19190 | 8 | 14 | 8 | 22 | +++ | +++ | *T.t.* 7/s. |
| 20646 | 17 | 10 | 9 | 10 | +++ | ++ | *T.t.* 5/s; *As.* few inf./s. |
| 19941 | 12 | 14 | — | — | +++ | +++ | *T.t.* < 1/s; *Str.* L 1. |
| 17314 | 20 | — | 9 | 19 | + | + | *T.t.* 2/s; *Str.* L 1; *As.* 10/s hw < 1/s. |
| 21068 | 11 | 12 | 7 | 3 | +++ | +++ | *T.t.* 2/s; *As.* 7/s. |
| 18415 | 12 | 7 | 12 | 10 | +++ | +++ | *T.t.* 6/s; *As.* 12/s. |
| 21713 | 15 | 5 | 12 | 8 | +++ | ++ | *T.t.* 49/s; *S.m.* |
| 18499 | 19 | 4 | 17 | 6 | ++ | − | *T.t.* < 1/s. |
| 20192 | 9 | 7 | 8 | 12 | +++ | + | *T.t.* < 1/s. |
| 22150 | 11 | 7 | 8 | 7 | +++ | ++ | *T.t.* < 1/s; *Str.* L 1, 2, 3; *As.* few inf. |

*T.t.* = *Trichuris trichiura.*
*As.* = *Ascaris lumbricoides.*
*S.m.* = *Schistosoma mansoni.*
*Str.* L 1, 2, 3 = first, second and third stage larvae of *Strongyloides stercoralis.*

*Trichuris* egg counts in the study group ranged from <1/smear to >100/smear. When eggs were numerous and distributed unequally in bunches or strands, counts were not attempetd. Reliable average counts were above 20/smear in only 3 (approximately 25, 50 and

TABLE 4 — *Number of individuals infected with various helminths, alone or in combinations*

| | | *T.t.* | *S.m.* | *Str.* |
|---|---|---|---|---|
| No helminths found | 1 | | | |
| *Taenia* only | 1 | | | |
| *Ascaris* only | 1 | | | |
| *Trichuris* only | 9 | 9 | | |
| *Trichuris, Taenia* | 3 | 3 | | |
| *Trichuris, S. mansoni* | 1 | 1 | 1 | |
| *Trichuris, Strongyloides* | 1 | 1 | | 1 |
| *Trichuris*, hookworm, *Strongyloides* | 1 | 1 | | 1* |
| *Trichuris*, hookworm, *S. mansoni* | 1 | 1 | 1 | |
| *Trichuris*, hookworm, *Taenia* | 1 | 1 | | |
| *Trichuris, Ascaris* | 10 | 10 | | |
| *Trichuris, Ascaris, S. mansoni* | 1 | 1 | 1 | |
| *Trichuris, Ascaris*, hookworm | 1 | 1 | | |
| *Trichuris, Ascaris, Strongyloides* | 1 | 1 | | 1* |
| *Trichuris, Ascaris, Strongyloides, S. mansoni* | 1 | 1 | 1 | 1 |
| *Trichuris, Ascaris, Strongyloides*, hookworm | 1 | 1 | | 1 |
| *Total* | *35* | *32* | *4* | *5* |

* Advanced-stage larvae in fresh stools and proctoscopic scrapings.
*T.t.* = *Trichuris trichiura.*
*S.m.* = *Schistosoma mansoni.*
*Str.* = *Strongyloides stercoralis.*

100 respectively). Counts were 5/smear to 10/smear in 6 and less than 5/smear in 20. Satisfactory material for egg counts was not obtained from 3 individuals. Among the 3 with moderate to heavy worm burdens (>20/smear), 2 also had *S. mansoni* infections and one of these had *Strongyloides* in addition. Eight of the 9 individuals with considerable *Trichuris* infections were among those with high levels of eosinophilia of the bowel exudates, and in 7 of them Charcot-Leyden crystals were abundant in the stools at one time or another

during the periods of observation. Among 45 unselected hospitalized adult male Africans without amebae, *Trichuris* egg counts were above 20/smear (84 and 38) in 2, and 5-20/smear in 5. None had dysentery and 3 had no bowel complaints.

## COMMENT

The severity of amebic dysentery in the African, reported by Elsdon-Dew (9) and his associates, has been confirmed by this study. In the usual case massive numbers of motile amebae containing red blood-cells were readily found among other elements generally regarded as typical of the amebic dysentery stool. If only two types of dysentery, bacillary and amebic, were to be considered, the macroscopic and microscopic characteristics observed in nearly all of the 35 cases studied would indicate clearly the latter (Table 5). However, the prevalence of eosinophils and giant epithelial cells among the

TABLE 5 — *Frequency of elements occurring conspicuously in stools and proctoscopic scrapings from 35 individuals with amebic dysentery*

| | | |
|---|---|---|
| Gross blood and mucus | | 35 |
| Clumping of red blood cells | | 15 |
| Pyknotic bodies | | 34 |
| Tissue cells, variously degenerate | | 34 |
| Sloughed epithelium and mucosal glands | | 12 |
| Neutrophils | | 16 |
| Eosinophils | | 32 |
| Charcot-Leyden crystals | | 15 |
| Giant epithelial cells (free in stools) | | 12 |
| Helminths, *probably* significant: | | |
| *Trichuris trichiura* 20/s | 3 | 5 |
| *Strongyloides stercoralis* | 2 | |
| Helminths, *possibly* significant: | | |
| *Trichuris trichiura* 6–10/s | 6 | 11 |
| *Trichuris trichiura* (clumped) | 2 | |
| *Strongyloides stercoralis* (3–1) | 2 | |
| *Schistosoma mansoni* (4–3) [1] | 1 | |

[1] Less duplications above.

cellular elements of the bowel discharges and the presence of certain helminths potentially damaging to the colon, both of which often accompanied the amebic infections, may have special significance.

Stool eosinophilia was a striking feature in the present group of cases. In a study of amebic dysentery in the Eastern Mediterranean area, Anderson (2) also was much impressed by the prevalence of eosinophils in the stool. Others working in the same area, however, failed to remark on this feature (26, 27). Observers in the Philippines and elsewhere have likewise not associated stool eosinophilia with amebic colitis (3, 5, 7, 14, 15, 17, 24). In one instance the presence of eosinophils was interpreted as indicating the absence of amebic involvement and the presence of intestinal allergy (16). On the other hand, there has been general agreement on the frequent association of Charcot-Leyden crystals with amebic dysentery and it may therefore be assumed, by inference, that stool eosinophilia is a general but seldom mentioned characteristic of amebic dysentery. At any rate, in view of the present findings, it now becomes a question of some interest as to whether eosinophils ever are lacking in the exudates of typical amebic dysentery. In a recent study of rectal biopsies from 14 cases in Arkansas, striking numbers of eosinophils often were seen in the tissues surrounding the amebic lesions (21).

Boddington and Truelove (4), in a study of proctoscopic scrapings from 31 patients with ulcerative colitis, observed a marked increase in the number of eosinophils (up to 15-20 % of all leucocytes seen) in 26 of 62 specimens. Equally interesting are the «giant epithelial cells» which they found commonly in the more severe cases of ulcerative colitis. Their interpretations of the origin and morphology of these large bodies are not entirely clear, but it is probable that the objects described by them are the same enlarged, sloughed goblet cells which in the present study were seen free in the stools of one-third, and free or among other epithelial cells in proctoscopic scrappings in nearly all cases. These structures have not previously been reported in association with amebic dysentery, possibly because they are relatively inconspicuous in hematoxylin-stained smears. Their presence in ulcerative colitis and in amebic dysentery of types seen in the present study probably has no special etiological significance since classical ulcerative colitis has not been recognized in the population group from which the present series of patients was drawn (28).

In at least a small proportion of the 35 cases in the present series, colon-damaging helminths possibly contributed importantly to the clinical manifestations of the amebic infections. In 5 of them worms may have been the predisposing or even the primary cause of the dysentery. Altogether there were 16 who had either *Strongyloides, S. mansoni* or moderate to heavy *Trichuris* infections. On the other hand, there were 3 in whom no colon-damaging worms were found and 16 others whose *Trichuris* infections were apparently light. In these the character of the exudates, including prominent eosinophilia and frequent presence of giant epithelial cells, can better be attributed to the amebae, at least for the present. In Anderson's (2) report in which eosinophilia was described as being a prominent feature of the exudates in amebic dysentery, no mention was made of associated helminths. In the present study, sections of amebic lesions of the bowel and biopsies of non-ulcerated areas (to be reported later) showed markedly increased numbers of eosinophils in a high proportion of cases. Abundant eosinophils were recently observed in sections of an ameboma (12). However, these cells and Charcot-Leyden crystals also have been found associated with malignant and other non-infectious lesions of the colon (23, 24).

No firm conclusions can be drawn as regards the significance of the eosinophilia of the exudates associated with amebic dysentery in the African at Durban. It appears likely that if appropriate staining technics were used, equally prominent eosinophilia of the bowel exudate in amebic dysentery would be revealed in other geographic areas. Eventually, it may become clearer as to whether amebic invasion of the mucosa can alone cause massive accumulation of eosinophils, or whether some other pathological condition of the colon, itself responsible for the eosinophilia, is a predisposing factor inducing tissue invasion by amebae which in the normal bowel would live as harmless commensals outside the tissues.

## SUMMARY

Amebic dysentery, exceptionally severe and common in the African native in Durban, South Africa, was studied in 35 adult males. Examination of the microscopic elements of the bowel exudates in stools and proctoscopic scrapings, wet-fixed and Giemsa-stained, revealed conspicuous eosinophilia of the colon, not

associated with eosinophilia of the blood, in 32 of the 35 cases. Large ameba-like bodies, described by Boddington and Truelove (4) as «giant epithelial cells» and regarded by them as characteristic of the exudates in ulcerative colitis, were observed in nearly all cases, and were found to be derived from goblet cells. These bodies were observed free in the stools, and were numerous in one-third of the cases. The eosinophilia and indeed the dysentery might be interpreted as due to *Trichuris trichiura* or *Strongyloides stercoralis* infections in 5 cases, and possibly these or *Schistosoma mansoni* infections could be regarded as significant in 11 others. However, the exudates were rich in eosinophils and Charcot-Leyden crystals when helminths were few or apparently lacking, suggesting a true relationship between amebic invasion and eosinophilia of the colon. The cause and effect relationship between amebic invasion and eosinophilia of the colon is therefore not yet clear. It seems unlikely, though it is of course possible, that the occurrence of both conditions in almost all of the cases studied was incidental.

## BIBLIOGRAPHY


1. Anderson, H. H., Bostick, W. L., and Johnstone, H. G. — *Amebiasis, Diagnosis and Chemotherapy*. Charles C. Thomas, Springfield, 431 pp., 1953.
2. Anderson, John — «A study of dysentery in the field with special reference to the cytology of bacillary dysentery and its bearing on early and accurate diagnosis», *Lancet*, ii: 998-1002, 1921.
3. Bercovitz, Z. — «Studies in the cellular exudates of bowel discharges. III. The diagnostic significance of cellular exudate studies in chronic bowel disorders», *Ann. Int. Med.*, **14**: 1323-1340, 1941.
4. Boddington, M. M., and Truelove, S. C. — «Abnormal epithelial cells in ulcerative colitis», *Brit. Med. J.*, **1**: 1318-1321, 1956.
5. Callender, G. R. — «The differential pathology of dysentery», *Am. J. Trop. Med.*, **14**: 207-220, 1934.
6. Callender, G. R. — «Amebic dysentery. The exudate as a guide to treatment», *Am. J. Trop. Med.*, **15**: 189-195, 1935.
7. Callender, G. R., and Inmon, E. H. — «Diarrhea, enteritis and dysentery», *Army Med. Bull.*, **40**: 24-75, 1937.
8. Elsdon-Dew, R. — «Some aspects of amoebiasis in Africans», *S. Afr. Med. J.*, **20**: 580, 620, 1946.
9. Elsdon-Dew, R. — «Endemic fulminating amebic dysentery», *Am. J. Trop. Med.*, **29**: 337-340, 1949.
10. Elsdon-Dew, R. — «Further aspects of amoebiasis in Africans», *Central Afr. J. Med.*, **2**: 291-294, 1956.
11. Elsdon-Dew, R., and Freedman, L. — «Intestinal parasites in the Natal Bantu», *S. Afr. J. Clin. Sci.*, **3**: 59-65, 1952.
12. Ferrara, A., Tigano, F., and Servadei, B. — «Manifestazioni neoplastiformi dell'amebiasis intestinale», *Arch. Ital. Sci. Med. Trop. Parassit.*, **38**: 171-197, 1957.

13. Garfinkel, B. T., Alvarez, M., and Oseasohn, R. — «The role of *Endamoeba histolytica* and *Trichiuris trichiura* in bloody diarrhea of children in Puerto Rico», *Am. J. Trop. Med. Hyg.*, **3**: 985-987, 1954.
14. Haughwout, F. G. — «Observations on the interpretation of the microscopic picture in dysentery and other intestinal disorders», *Trans. V. Biennial Congr. Far East. Assoc. Trop. Med., Singapore 1923*, pp. 482-512, 1924.
15. Haughwout, F. G. — «The practical microscopic diagnosis of dysentery», *Bull. Inst. Med. Res. Fed. Malay States*, No. 3, Kuala Lumpur, 90 pp., 1924.
16 Haughwout, F. G. — «Some departures from the typical cell picture in bacillary and amebic dysentery — observations on the presence of eosinophils in intestinal allergy», *Philippines J. Sc.*, **25**: 513, 1924.
17. Haughwout, F. G. — «Dysentery, colitis and diarrhea in Japanese civilian prison camps in the Philippines during World War II. I. Primary, concurrent and recurrent dysentery», *Am. J. Dig. Dis.*, **15**: 142-150, 1948.
18. Hornung, Maria — *Charcot-Leyden crystals. Isolation, concentration, preservation and preliminary biochemical studies.* Dissertation, Tulane University, 1958.
19. Jung, R. C., and Beaver, P. C. — «Clinical observations on *Trichocephalus trichiurus* (whipworm) infestation in children», *Pediatrics*, **8**: 548-557, 1951.
20. Jung, R. C., and Jelliffe, D. B. — «The clinical picture and treatment of whipworm infection», *West Afr. Med. J.*, **1**: 11-15, 1952.
21. Juniper, K., Steele, V. W., and Chester, C. L. — «Rectal biopsy in the diagnosis of amebic colitis», *Southern Med. J.*, **51**: 545-553, 1958.
22. Kessel, J. F. — «The distinguishing characteristics of the intestinal protozoa of man», *China Med. J.*, **39**: 85-122, 1925.
23. Manson-Bahr, P. — «Facts and fallacies in the diagnosis of amebiasis», *Central Afr. J. Med.*, **1**: 269-279, 1955.
24. Manson-Bahr, P., and Muggleton, W. J. — «Rectal biopsy as an aid to the diagnosis of amoebic dysentery and allied diseases of the colon», *Lancet*, Apr. 13, p. 763, 1957.
25. Randolph, R. G. — «Differentiation and enumeration of eosinophils in the counting chamber with a glycol stain; a valuable technic in appraising ACTH dosage», *J. Lab. Clin. Med.*, **34**: 1696-1701, 1949.
26. Thomson, J. G., and Robertson, A. — «The value of laboratory reports on stools in cases of suspected amoebic dysentery, and their interpretation by the clinician; with a special note on the diagnostic significance of Charcot-Leyden crystals», *Proc. Roy. Soc. Med. (Sec. Trop. Dis. Parasitol.)*, **14**: 33-44, 1921.
27. Willmore, J. G., and Shearman, C. H. — «On the differential diagnosis of the dysenteries. The diagnostic value of the cell-exudate in the stools of acute amoebic and bacillary dysentery», *Lancet*, II: 200-206, 1918.
28. Wilmot, A. J. — Personal communication.
29. Wilmot, A. J., Armstrong, T. G., and Elsdon-Dew, R. — «The comparative value of amoebicidal drugs», *J. Trop. Med. Hyg.*, **54**: 161-165, 1951.

# EXPERIMENTAL INTESTINAL AMEBIASIS

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Much effort has been expended and gratifying progress has been made during the past twenty years toward technics for inducing intestinal amebiasis in animals. However, much less progress has been made in understanding the precise determinants of infection. Time limitations restrict this review to only certain types of experimentally induced intestinal infections, although a broad review of the problem would also include consideration of the naturally acquired disease in animals.

## DOGS

This was the first experimental host. Lösch (1875), who first recognized *Entamoeba histolytica,* induced intestinal amebiasis in a dog. However, he had such erratic success — only one of 4 dogs became infected in his series — that he doubted the role of amebae in pathogenesis. Subsequent workers (see Faust, 1930, 1932*a, b,* for a literature review) also had indifferent results and it was generally accepted that dogs were unsuited for experimental amebiasis.

Fortunately, reinvestigation of this animal by Faust (1930, 1931, 1932*a, b*) and his colleagues (Faust and Kagy, 1934*a, b*) during the 1930's (see Faust, Carrera, and Bradin, 1953, for a review) demonstrated the dog to be a most useful host. This change of view apparently stemmed from such factors as the use of highly virulent strains, adherence primarily to the intrarectal route of inoculation which earlier workers (see Faust, 1930) had found to be best, and

recognition of the importance of diet. They found that the infection was enhanced on a diet of salmon or ventriculin (desiccated pigs stomach) while diets containing substantial amounts of liver or cod liver oil ameliorated the infection. They were unable to explain these effects satisfactorily, and the speaker (unpublished) was unable to detect any consistent qualitative or quantitative change in the enteric bacteria, but he did find that canned salmon consistently induces a mild diarrhea in normal dogs. The chief subsequent use of these observations on the effects of the diet seems to have been the utilization of salmon. Schwartzwelder and Avant (1952) used canned salmon intermittently to exacerbate infections, and Thompson *et al.* (1949, 1950, 1954, 1955*a*, *b*, *c*, 1956, 1958) have fed canned salmon continuously so as to maintain uniformly severe infections for chemotherapeutic studies. Most workers have used young dogs, but a careful study of the effect of age on susceptibility does not seem to have been made.

The following account summarizes the speaker's experience *(loc. cit.)* with dogs. Some 400 dogs have been infected with strain 200 *E. histolytica* and observed for periods ranging from a few days to over a year. The work covered a continuous period of 10 years with about 15 dogs under observation at a time. Small young dogs of either sex and of various breeds are observed for health for two weeks on conventional food before they are placed on a strict diet of canned salmon, supplemented with cod liver oil and brewer's yeast. They are examined twice by colonic irrigation as a step toward excluding pre-existing infection with amebae and enteritis and then they are inoculated intra-rectally with several million amebae from cultures or a donor dog. Fresh colonic aspirates are examined for amebae one to three times weekly thereafter.

Approximately half of the dogs show patent infections within a week; Faust (1932) reported 73 % of 77 dogs became infected after a single inoculation. Animals negative at one week are reinoculated at weekly intervals until they become infected or until 5 trials have been made; only about 3 % are refractory to these procedures. The infection is predominantly subacute, characterized by numerous trophozoites in dysenteric stools; amebae are readily demonstrable in 85 % to 90 % of the samples. The infections typically last for many months; spontaneous recovery within one month has occurred in only

3 % of the dogs (Table 1). Infection induces only a low grade resistance to reinfection. Although infected dogs lose weight and become anemic, they rarely die; this low mortality is in contrast to Faust's (1932) report of more than a 50 % mortality due to amebic enteritis. Amebic liver abscess occurs only rarely (see also Faust and Kangy, 1934*a*). Strain 200 amebae in salmon-fed dogs provides a notably useful host-parasite relationship, which is characterized by low host mortality yet remarkably stable patent infections.

TABLE 1 — *Stability of untreated intestinal amebiasis in 382 experimentally infected dogs* [1]

| Days of observation | Number of dogs | | |
|---|---|---|---|
| | Observed | Retained infection | Lost infection [2] |
| 1–10 . . . . . | 99 | 99 | 0 |
| 11–20 . . . . . | 180 | 178 | 2 |
| 21–40 . . . . . | 55 | 55 | 0 |
| 41–80 . . . . . | 30 | 29 | 1 |
| 81–160 . . . . . | 10 | 9 | 1 |
| 161–320 . . . . . | 5 | 5 | 0 |
| > 320 . . . . . | 3 | 2 | 1 |

[1] Infected with strain 200 *E. histolytica* and maintained on canned salmon supplemented with cod liver oil and brewer's yeast. Among 390 dogs 12 (3.1 %) were refractory to 5 inoculations.

[2] On basis of 12 consecutively negative samples of colonic aspirate evenly spaced during one month.

Cod liver oil and yeast are needed to compensate for the low levels of vitamin A and of B-complex vitamins. Since the foregoing results were obtained with a diet that is made rich in nicotinic acid by including yeast, it is apparent that a nicotinic acid deficiency is not essential for good infectivity, even though Larsh (1952) has concluded that it lowers resistance. Except for deficiencies in iron, calcium, and phosphorus, canned salmon supplemented with cod liver oil and yeast provides an adequate diet for the dog insofar as its nutritive requirements are known (see Spector, 1957). Recent unpublished work by the speaker and his associates has shown that supplemen-

tation of the diet with the needed amounts of iron, calcium, and phosphorus does not alter the stability of the infections.

## KITTENS

The speaker has had no personal experience with this animal and time does not permit review of the vast literature on its use. Therefore, the kitten will be discussed only sufficiently to place it in perspective relative to other experimental hosts. Dating from the latter part of the 19th century (see Dale and Dobell, 1917, for early references), kittens have been known to be extremely susceptible to amebic infection, particularly following the direct implantation of trophozoites into the lower bowel. Numerous workers have agreed that older animals are more resistant (cf. Dale and Dobell, *loc. cit.*, Clampit, 1948). Infected kittens develop a severe dysentery and usually die within two days to two weeks; bacteria play a prominent role in causing death, as manifested by septicemia, bronchopneumonia, and the type of intestinal pathology. Dietary manipulations are not necessary for establishing the infection and apparently the effects of diet have not been studied to any appreciable extent. Bacteria are capable of producing dysenteric symptoms in kittens that simulate amebic dysentery (see references in Hoare and Neal, 1955). Amebic abscess of the liver is a rare complication of amebic dysentery in kittens (Dale and Dobell, 1917; Carrera, 1950).

## RATS

The use of this animal goes back to Lynch (1915), but subsequent workers during the next three decades (see Jones, 1948, for references) either achieved infection only occasionally or failed entirely. Jones (1946) reinvestigated this animal and showed that young rats could be infected readily. His success derived from the use of weanling rats, amebae of known virulence, and intracecal inoculation. Jones (1946, 1948) did not specify the diet used and the only subsequent workers that have provided a basis for appraising the effect of diet were Taylor *et al.* (1952). Upon learning of the successful

use by the speaker of a low residue, rich protein, and rich carbohydrate diet that was developed for breeding rats, they found that the rat breeder diet gave much more severe infections than commercial rat pellets. The factors responsible for this effect are unknown.

Weanling rats also may be infected orally with cysts. McCowen and Lawlis (1950) and McCowen *et al.* (1953) were able to induce infections rather consistently by the stomach tube administration of 50,000 to 100,000 strain 200 cysts to rats that had been preconditioned for 48 hours on a diet containing 90 % salmon.

Mention should be made here that many colonies of rats are naturally infected with *E. muris* which can serve as a complicating factor (cf. Neal, 1949) in using this host.

TABLE 2 — *Diet for intestinal amebiasis in rats* [1]
(Percentage composition)

| | |
|---|---|
| Whole corn meal | 33 |
| Ground whole wheat | 31 |
| Whole milk powder | 21 |
| Linseed oil meal | 7 |
| Dried hog liver | 3 |
| Alfalfa | 2 |
| Brewer's yeast (G) | 2 |
| Sodium chloride | 0.5 |
| Sodium carbonate | 0.5 |

[1] Thompson *et al.*, 1950.

The remaining comments on intestinal amebiasis in rats will be drawn from observations made by the speaker and his associates (Thompson *et al., loc. cit.*) in work involving approximately 45,000 animals during the past 10 years. Twenty- to 22-day old albino rats are used from a colony that regular sampling has demonstrated to be essentially free of *E. muris*. The animals are maintained entirely on water and the diet shown in Table 2. Infections are induced intracecally with 5,000 to 100,000 trophozoites of strain 200 *E. histolytica*. The amebae are harvested from 24-hour, rapidly multiplying cultures, and the inoculum is adjusted according to the virulence of the strain. A relationship between the number of amebae injected and both the incidence and severity of infection has been observed repeatedly. Cecal

ulceration occurs within 24 hours and infections persist through at least 3 weeks after inoculation (Table 3). Strain 200 cultures predictably decline in virulence during 2 years of subcultivation (Thompson *et al.*, 1954); most of this decline can be compensated for by increasing the inoculum, but it eventually becomes expedient to shift to a

TABLE 3 — *Course of intestinal infection in rats* [1]
(10–12 rats per group were infected from a common pool of 200 B amebas on 10/27/1952)

| Days after inoculation | ADI | % inf. |
|---|---|---|
| +1 | 2.2 | 90 |
| 3 | 3.7 | 100 |
| 5 | 3.0 | 90 |
| 7 | 2.7 | 80 |
| 9 | 3.3 | 80 |
| 11 | 3.1 | 90 |
| 13 | 2.6 | 70 |
| 15 | 2.9 | 80 |
| 17 | 3.6 | 80 |
| 19 | 2.1 | 75 |

[1] Thompson *et al.*, 1954.

line that has been recently passed through dogs. The bacterial associates of each line are shown to be innocuous for rats before the passaged amebae are used. Vigorously growing, virulent cultures and a favorable host diet appear to have been the key determinants in the success of this work.

## GUINEA PIGS

Although a number of investigators had worked with this animal (see Taylor *et al.*, 1950, for a literature review), successful use of guinea pigs stems from the work of Carrera and Faust (1949). Infections are induced by injecting trophozoites intracecally following laparotomy. Infections are induced more readily by injecting trophozoites intracecally than by administering cysts orally (Sadun *et al.*, 1950).

An effect of diet on susceptibility has been observed (Taylor *et al.*, 1950, 1952; Sadun *et al.*, 1951; Lynch, 1957); an ascorbic acid deficiency increases susceptibility (Sadun *et al.*, 1951), but is not necessary for infection. Conventional balanced guinea pig diets are less favorable to infection than rabbit chow plus kale or several synthetic diets. The only solid clue as to how an adequate diet influences susceptibility has been provided by Lynch (1957); he found that an infection-enhancing synthetic diet caused marked atrophy of the cecal mucosa, and he postulated that this lowered resistance to amebic and bacterial invasion. High susceptibility of the guinea pig to intestinal amebiasis is indicated by severe dysentery, high mortality, and hepatic invasion from the intestinal tract (Rees *et al.*, 1954; Bock and Mudrow-Reichnow, 1955; Krup, 1956). However, sufficient proliferation to produce a liver abscess is essentially unknown. Phillips *et al.* (1955) were unable to induce ulcerative intestinal amebiasis in guinea pigs in the absence of bacteria. Although most workers have used young guinea pigs, the effect of age on susceptibility has not been adequately characterized.

## RABBITS

Tobie (1949) reviewed the early, essentially futile efforts to use this animal and described his own successful work with this species. Hunninen and Boone (1957) also have found the rabbit to be useful. Tobie *(loc. cit.)* reported that rabbits fed a grain-bread ration were highly susceptible to intracecal inoculation with a virulent line of strain 200 amebae. Infections were induced less regularly when cysts of these amebae were given *per os*. The effects of diet and of age on susceptibility have not been studied sufficiently for definitive statements concerning their influence. Infections in rabbits seem to approach those in guinea pigs with regard to severity and mortality.

## CONCLUDING STATEMENT

Time limitations preclude a more detailed review of the pathology and other characteristics of experimental infections.

Animal infection models of human amebic dysentery probably are now available in a sufficient variety of hosts, but adequate exper-

imental models of chronic human amebiasis are laking. There are some indications that certain species of monkeys may be suitable hosts for experimental chronic amebiasis (cf. Miller, 1952, for a review), but additional work would be needed to determine their usefulness. Experimental infections provide a useful means not only for developing better therapeutic and prophylactic drugs, but also for gaining a better understanding of the determinants of amebiasis.

## BIBLIOGRAPHY


Bock, M., and Mudrow-Reichenow, L. — *Ztsch. Tropenmed. & Parasit.*, **6**: 344, 1955.

Carrera, G. M., and Faust, E. C. — *Am. J. Trop. Med.*, **29**: 647, 1949.

Carrera, G. M. — *Arch. Path.*, **50**: 1, 1950.

Clampit, J. M. — *Am. J. Trop. Med.*, **28**: 289, 1948.

Dale, H. H., and Dobell, C. — *J. Pharmacol. & Exp. Therap.*, **10**: 399, 1917.

Faust, E. C. — *Proc. Soc. Biol. & Exp. Med.*, **27**: 908, 1930.

Faust, E. C. — *Puerto Rico J. Pub. Hlth. & Trop. Med.*, **6**: 391, 1931.

Faust, E. C. — *Am. J. Trop. Med.*, **12**: 37, 1932*a*.

Faust, E. C. — *Proc. Soc. Exper. Biol. & Med.*, **29**: 659, 1932*b*.

Faust, E. C., and Kagy, E. S. — *Am. J. Trop. Med.*, **14**: 221, 1934*a*.

Faust, E. C., and Kagy, E. S. — *Am. J. Trop. Med.*, **14**: 235, 1934*b*.

Faust, E. C., Scott, L. C., and Swartzwelder, J. C. — *Proc. Soc. Exper. Biol. & Med.*, **32**: 540, 1934.

Faust, E. C., Carrera, G. M., and Bradin, J. L. — *Bull. Tulane Un. Med. Faculty*, **12**: 62, 1953.

Hoare, C. A., and Neal, R. A. — *Symposia Soc. Gen. Microbiol. V. Mechanismes of Microbial Pathogenicity*, 230, 1955.

Hunninen, A. V., and Boone, H. A. — *Am. J. Trop. Med. & Hyg.*, **6**: 32, 1957.

Jones, W. R. — *Ann. Trop. Med. & Parasitol.*, **40**: 130, 1946.

Jones, W. R. — *Proc. 4th Int. Cong. Trop. Med. & Mal.*, 1088, 1948.

Kagy, E. S., and Faust, E. C. — *Proc. Soc. Exper. Biol. & Med.*, **28**: 252, 1930.

Krupp, I. M. — *Exper. Parasitol.*, **5**: 421, 1956.

Larsh, Jr., J. E. — *Am. J. Trop. Med. & Hyg.*, **1**: 970, 1952.

Lösch, F. — *Arch. Path. Anat.*, **65**: 196, 1875.

Lynch, J. E. — *Am. J. Trop. Med. & Hyg.*, **6**: 813, 1957.

Lynch, R. M. — *J. A. M. A.*, **65**: 2232, 1915.

McCowen, M. C., and Lawlis, Jr., J. F. — *J. Parasitol.*, **36**: 26, 1950.

McCowen, M. C., Callender, M. E., Lawlis, Jr., J. F., and Brandt, M. C. — *Am. J. Trop. Med. & Hyg.*, **2**: 212, 1953.

Miller, M. J. — *Am. J. Trop. Med. & Hyg.*, **1**: 417, 1952.

Neal, R. A. — *Nature*, **163**: 99, 1949.

PHILLIPS, B. P., WOLFE, P. A., REES, C. W., GORDON, H. A., WRIGHT, W. H., and REYNIERS, J. A. — *Am. J. Trop. Med. & Hyg.*, **4**: 675, 1955.

REES, C. W., TAYLOR, D. J., and REARDON, L. V. — *J. Parasitol.*, **40**: 390, 1954.

SADUN, E. H., CARRERA, G. W., KRUPP, I. M., and ALLAIN, D. S. — *Proc. Soc. Exper. Biol. & Med.*, **73**: 362, 1950.

SADUN, E. H., BRADIN, JR., J. L., and FAUST E. C. — *Am. J. Trop. Med. & Hyg.*, **31**: 426, 1951.

SPECTOR, W. S. — *Handbook of Biological Data*. W. B. Saunders Co., Philadelphia, 1956.

SWARTZWELDER, C., and AVANT, W. H. —*Am. J. Trop. Med. & Hyg.*, **1**: 567, 1952.

TAYLOR, D. J., GREENBERG, J., HIGHMAN, B., and COATNEY, G. R. — *Am. J. Trop. Med. & Hyg.*, **30**: 817, 1950.

TAYLOR, D. J., GREENBERG, J., and JOSEPHSON, E. S. — *Am. J. Trop. Med. & Hyg.*, **1**: 559, 1952.

THOMPSON, P. E., and LILLIGREN, B. L. — *Am. J. Trop. Med. & Hyg.*, **29**: 323, 1949.

THOMPSON, P. E., DUNN, M. C., BAYLES, A., and REINERTSON, J. W. — *Am. J. Trop. Med. & Hyg.*, **30**: 203, 1950.

THOMPSON, P. E., MCCARTHY, D., and REINERTSON, J. W. — *Am. J. Hyg.*, **59**: 249, 1954.

THOMPSON, P. E. — *Antibiotic Med.*, **1**: 603, 1955*a*.

THOMPSON, P. E., REINERTSON, J. W., MCCARTHY, D. A., BAYLES, A., and COOK, A. R. — *Antibiotics & Chemotherapy*, **5**: 433, 1955*b*.

THOMPSON, P. E., REINERTSON, J. W., BAYLES, A., MCCARTHY, D. A., and ELSLAGER, E. F. — *Am. J. Trop. Med. & Hyg.*, **4**: 224, 1955*c*.

THOMPSON, P. E., MCCARTHY, D. A., BAYLES, A., REINERTSON, J. W., and COOK, A. R. — *Antibiotics & Chemotherapy*, **6**: 337, 1956.

THOMPSON, P. E., MCCARTHY, D. A., REINERTSON, J. W., BAYLES, A., and NAJARIAN, H. —*Antibiotics & Chemotherapy*, **8**: 37, 1958.

TOBIE, J. E. — *Am. J. Trop. Med.*, **29**: 859, 1949.

# EXPERIMENTAL HEPATIC AMOEBIASIS

BRIAN MAEGRAITH
and
CHAMLONG HARINASUTA

Consideration of the development of intestinal lesions in the guinea pig caecum following direct introduction of *E. histolytica* trophozoites is necessary for an understanding of the production of liver abscesses with the same parasite strain, since such abscesses can be easily and constantly induced *only* in animals in which chronic intestinal lesions have already been established (Maegraith and Harinasuta, 1953, 1954*a*, *b*).

## THE PRODUCTION OF INTESTINAL LESIONS

The guinea pig is very susceptible to intracaecal injection of suitable strains of *E. histolytica* (Taylor *et al.*, 1950). We used an inoculum of 1.0 million washed trophozoites grown in preconditioned LES medium for 48 hours at 37°C and freshly suspended in physiological saline, injected (under ether anesthesia) into the lumen of the caecum at a point about half an inch from the termination of the ileum.

*a*) Acute lesions in the caecal mucosa developed in 60 % to 90 % of animals in 7-14 days. Better results were obtained with trophozoites washed in saline suspension than with trophozoites unwashed or suspended in LES overlay. We considered that the horse serum in the medium may have had some inhibitory effect on the initial development of the lesion. Lesions were found at the site of injection, in isolated areas some distance from it, and occasionally in the ileal mucosa.

*E. histolytica* was cultured from scrapings of lesions and from the faeces of animals which developed lesions, but not from those which did not.

The development of the acute intestinal lesion was followed histologically and by culture. Certain clearly defined stages were observed (Torpy and Maegraith, 1956). The earliest sign of invasion of the mucosa was seen in the «occupation» of the epithelium by amoebae and associated local colonization (Fig. 1).

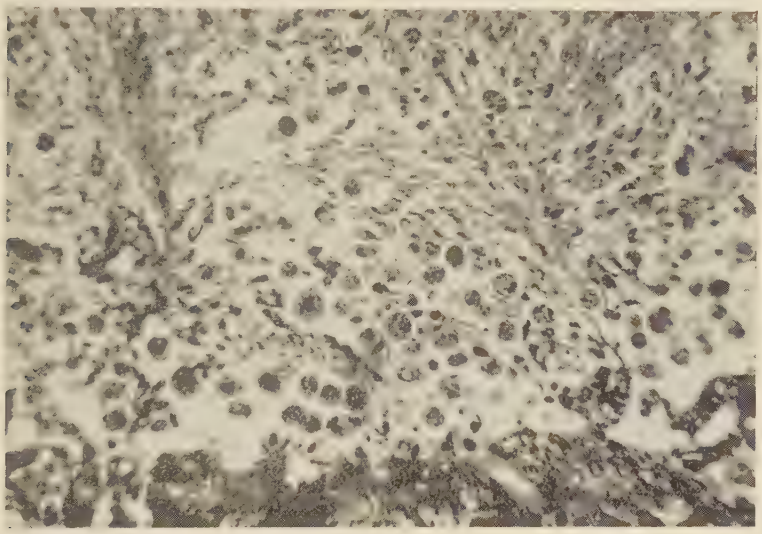

Fig. 1 — Acute intestinal lesion in guinea pig. First colonization of *E. histolytica* at surface of caecum

The mechanism of this invasion was not determined. Evidence of enzyme activity was obtained in that the invading strains possessed gelatinase (Harinasuta and Maegraith, 1954), but we have not been able to satisfy ourselves that the proteins and other components of epithelium itself are directly involved, nor have we been able to determine whether the gelatinase is inherent in the cellular body of the parasite or synthesized by it in a suitable environment. (It would be very useful to pursue this point further, since it might help to differentiate «pathogenic» strains from others or from themselves in the passive state. The fact is that we do not seem to know very much

about the meaning of pathogenesis or virulence in relation to *E. histolytica* since definitions based on animal experiments all seem to break down from time to time. For instance, a recent strain originally active in both guinea pigs and rats is now still active in the former but not in the latter.)

Fig. 2 — Acute intestinal lesion in guinea pig. First break-through *muscularis mucosae*

Breakdown of the epithelial surface quickly led to ulceration. The parasite in 24 to 48 hours penetrated with further colonization to the *muscularis mucosae,* below which large numbers of lymphocytic cells and a few eosinophils and polymorphs accumulated. This barrier was broken in isolated areas in which the amoebae were concentrated (Figs. 2 and 3). The lesion so formed in the submucosa was at first walled in by cellular infiltration, with an advancing «front» containing many amoebae. Later, the amoebae and accompanying cellular reactions reached the muscular layers and even the subperitoneal tissue.

We were unable to determine whether the point of first invasion of the mucosal epithelium was related to the mucous glands, but staining reactions indicated that the areas involved were mucus-free.

The distribution of lesions over the caecal mucosa occurred by direct spread in many cases, but evidence of other modes of extension was present. Lesions for instance developed inches away from the point of injection of the parasites, tending especially to appear in the

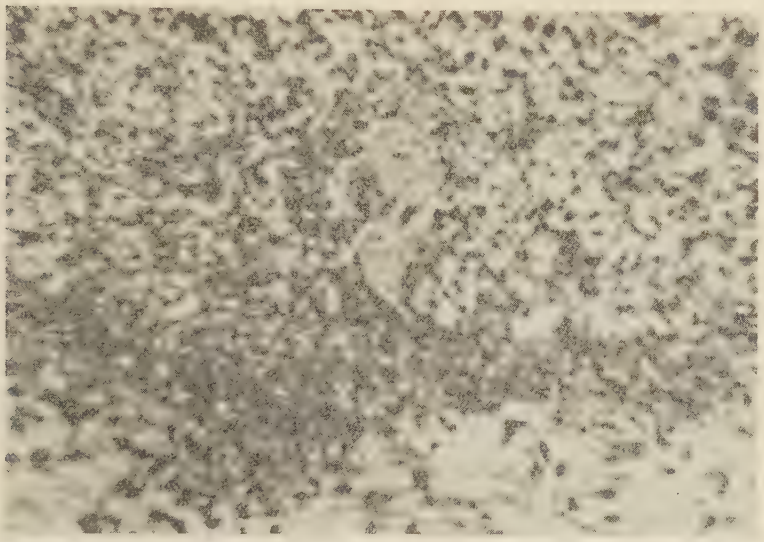

Fig. 3 — Acute intestinal lesion in guinea pig. As Fig. 2: high power view of advancing edge of break-through, showing *E. histolytica*

lymphatic nodules, which were usually swollen and very prominent. Serial section of the nodules sometimes showed trophozoites of *E. histolytica* deeply imbedded in the tissue, suggesting that some of the more distal lesions at any rate were not derived from direct invasion from parasites in the gut lumen, or from direct lateral extension, but from lymphatic distribution (Fig. 4). Attempts were made to confirm this by injections of India ink into the normal and infected gut wall and later by direct injection of amoebae into areas of the wall known to contain the larger lymphatic channels. In the latter case, lesions could sometimes be induced at some distance from the point of injection, without injection of amoebae into the lumen. Lymphatic spread may therefore be an important mode of extension of amoebic lesions in the gut wall.

*b*) Chronic intestinal lesions were easily produced by alternate infection and incomplete treatment with diodoquine (Maegraith and Harinasuta, 1953). These lesions were massive, ulcerative and associated with extensive repair processes at the periphery, frequently extending into the muscle layers and to the peritoneal surface. The

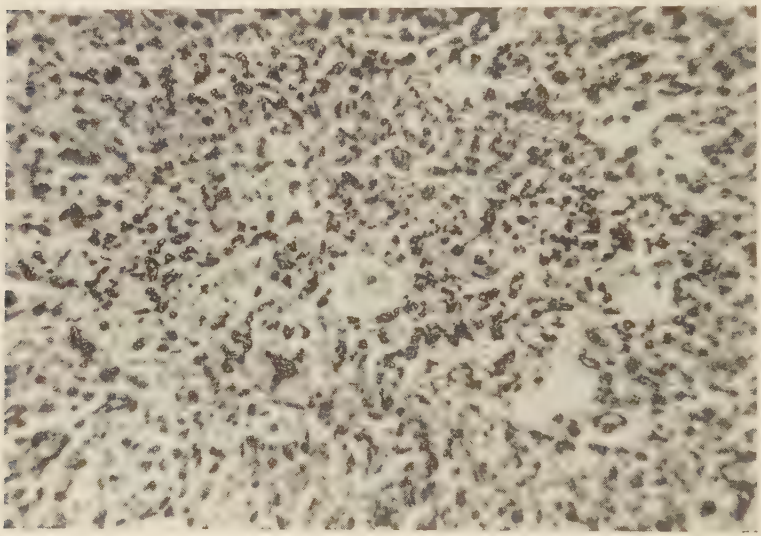

Fig. 4 — Acute intestinal lesion in guinea pig. *E. histolytica* in lymphatic nodules remote from site of injection and subsequent lesions

significance of such lesions lay in the relation between their presence and the ease with which hepatic lesions could be produced by intraportal injection of homologous *E. histolytica* trophozoites.

## THE PRODUCTION OF HEPATIC LESIONS

As reported elsewhere (Maegraith and Harinasuta, 1954*b*), we studied the results of injection of *E. histolytica* trophozoites into the portal vein.

In normal guinea pigs injection of trophozoites into either the mesenteric vein or branches of the portal vein (usually the right) resulted consistently in the appearance of abscesses scattered or localized in the right lobes. For the first day or two amoebae could be identified in and sometimes cultured from these lesions, but, without exception, rapid recession occurred and in most cases healing was advanced by the fourth day (Fig. 5).

Fig. 5 — Intraportal inoculation of *E. histolytica:* healed abscesses in right lobes 4 days after inoculation of parasites (normal animal)

Intraportal injection of suspensions of the same strain of *E. histolytica* (5 in all) into animals of the same weight in which active «chronic» intestinal lesions had been induced led to the development of persistent hepatic abscesses, which sometimes involved practically the whole of the right lobe of the liver, and from which amoebae could be recovered 10 days or more after the injection. These abscesses in many ways resemble those seen in natural infections in man (Fig. 6).

Unfortunately, however, the suspensions of *E. histolytica* used in our experiments were not bacteria-free, although the organisms concerned were antibiotic sensitive and could thus be largely controlled. Undoubtedly, this bacterial element complicates the picture and must have played a part in the histological patterns finally achieved, including the common peripheral infiltrations of lymphocytes and

Fig. 6 — Intraportal inoculation of *E. histolytica*. Persistent abscess in right lobe after 14 days. Animal with chronic intestinal lesion

polymorphs and later formation of fibrous «capsules» about some abscesses. Nevertheless, the bacteria were common to all groups of animals in the experiments, normal or with «chronic» gut infections, and we consider we are justified in assuming that the successful production of persistent liver lesions in the latter, and the failure to do so in the former indicate the significance of the presence of the gut lesion.

The development of the liver lesion was studied in both groups of animals.

In *normal* animals a few hours after the injection small periportal lesions had developed, with polymorph and lymphocytic infiltration and some local parenchymal degeneration and necrosis. These lesions were usually isolated, occasionally confluent. Amoebae could sometimes be seen in the relevant branch of the portal vein (Fig. 7). Small necrotic *foci* involving groups of portal tracts and associated paren-

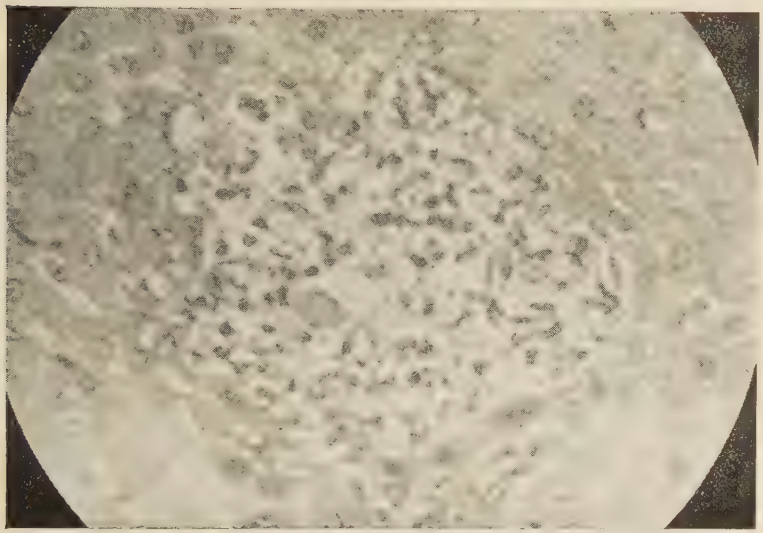

Fig. 7 — Intraportal inoculation of *E. histolytica*. *E. histolytica* in portal venule (normal animal after 7 thours)

chyma with moderate mixed cell infiltration developed inside 24 hours, at which point the trophozoites were visible in the small portal vessels and could occasionally be cultured (Fig. 8). At 72 hours abscesses had formed containing necrotic cellular material, polymorphs, lymphocytes and bacteria and surrounded by necrosing parenchymal cells and infiltrations of polymorphs and lymphocytes. Beyond the wall of the main abscess there were sometimes scattered discrete periportal lesions. Healing began after this, and was rapid, with some fibrosis of the area involved, including the smaller periportal lesions (Fig. 9). Amoebae could not be cultured or seen after the third day.

In animals with «chronic» intestinal lesions the picture in the early stages was similar to that observed in normal animals, but was more vigorous and advanced. Within 24 hours there was irregular occlusion of the small radicles of the portal vein with emboli containing amoebae. The affected tracts were infiltrated with polymorphs and lymphocytes, extending into the lobular tissue, with degeneration and necrosis of periportal and adjacent parenchymal cells. Areas of focal necrosis spread to form larger *foci* which developed into smal

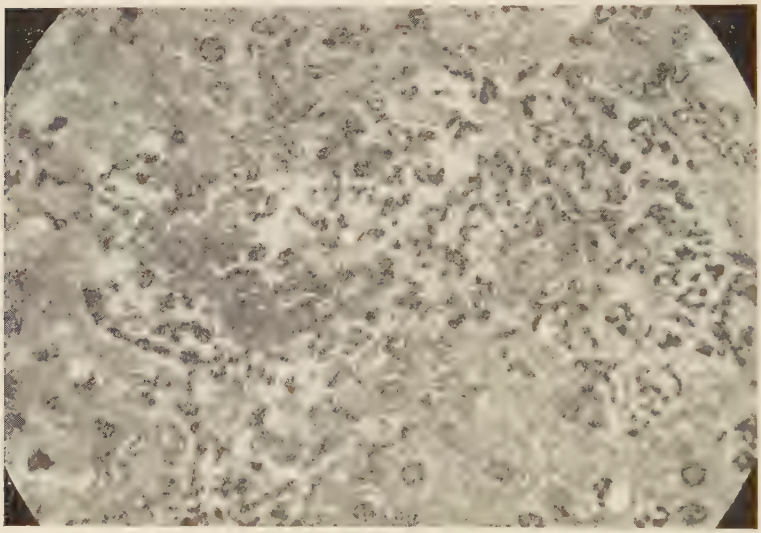

Fig. 8 — Intraportal inoculation of *E. histolytica*. Periportal lesion (normal animal after 7 hours)

abscesses, containing granular debris, lysed and necrotic epithelial cells and mixed polymorphs and round cells; there were occasional amoebae and masses of bacteria. Areas of infarction containing periportal lesions were common. Distant parenchymal cells often showed fatty degeneration; congestion of central veins and sinusoids in nearby lobules was notable, and there was later some fibroblastic response in the surrounding tissue.

By the end of the second day destruction of liver tissue was more pronounced and large abscesses (sometimes including practically the whole of the right medial lobe) developed by the third or fourth day. From then to the twelfth day, confluent abscesses were present, in the periphery of which *E. histolytica* could be demonstrated and cultured. Repair at the periphery started about the fourth day. Some

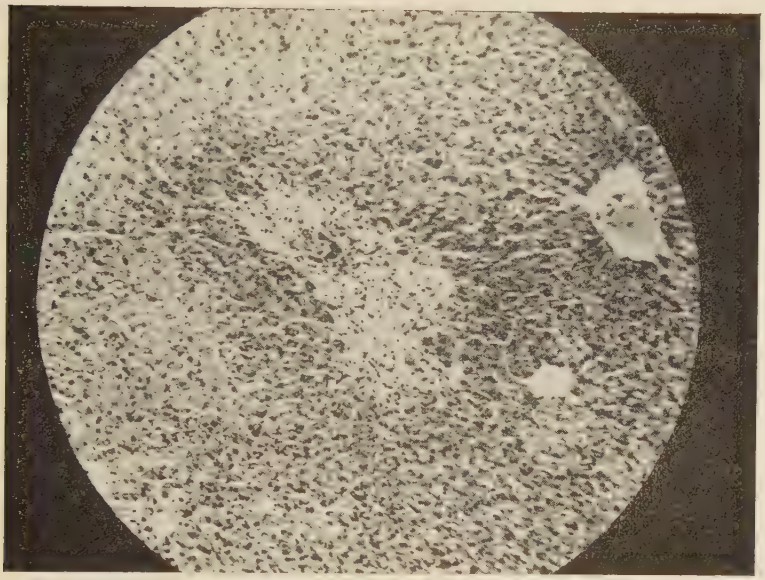

Fig. 9 — Intraportal inoculation of *E. histolytica*. Healed periportal lesion (normal animal after 4 days: same animal as Fig. 5)

abscesses were eventually completely walled off by bands of fibrous tissue, but in a few there was no fibroblastic response and the line of demarcation between the abscess and surrounds was a narrow congested zone of necrotic cells, infiltration and amoebae.

In some of the normal animals extensive areas of cellular degeneration and necrosis involving all local tissues in many adjacent lobules were observed. We subsequently discovered that similar lesions, which appeared to be infarcts, could be induced by intraportal injection of inert particles, including rice starch granules. In the

experimental animals the infarcts were sharply demarcated and were sometimes involved in abscess formation (Fig. 10). Some of these infarcted areas had engulfed scattered periportal lesions, and it seems likely that the larger abscesses may have evolved in this way in infarcts rather than by coalescence of embolized portal venous radicles, as suggested by Anderson *et al.* (1953). In support of this contention, we found that large abscesses were uncommon after injection of

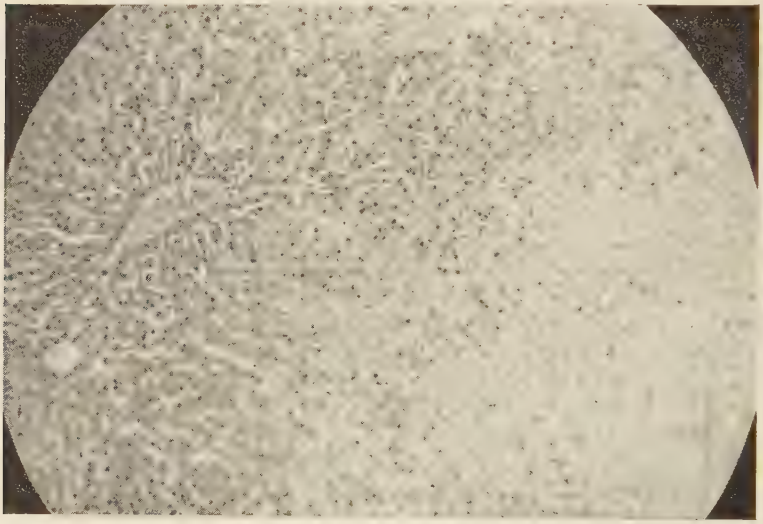

Fig. 10 — Intraportal inoculation of *E. histolytica*. Epatic infarct (animal with chronic intestinal lesion, 48 hours after inoculation)

amoebae into the mesenteric vein, which procedure seldom gave rise to infarcts. The speed of production of the major lesions is also in favour of their having been extensive from the beginning.

Areas of infarction were common in the «chronic» intestinal group of animals and usually contained many periportal lesions which had presumably been established before the failure of the blood supply to the larger area. These infarcted areas were again the seat of later large abscesses.

Although some authors have stated that infarcts are not easy to produce in the liver, owing to the dual blood supply (Boyd, 1953),

we have had no difficulty in either the guinea pig or the dog. In these and other animals the blood reaching the lobules is largely mixed venous and arterial, and portal-hepatic arterial and hepatic arterial-hepatic venous shunts have been demonstrated (Andrews *et al.*, 1949; Maegraith, 1953; Andrews and Maegraith, 1953). The development of infarcts following introduction of material into the portal vein is therefore not surprising since the vascular arrangements are such that infarcts could arise from occlusion of the portal vein itself or of the hepatic artery or vein.

## DISCUSSION

In normal animals trophozoites of *E. histolytica,* when injected *via* the portal vein, get caught up in the smaller branches of the portal vein, where they settle temporarily, and may give rise to lesions and even abscesses which, however, do not persist but heal rapidly and are replaced in a few days by fibrous tissue.

In animals with chronic intestinal lesions similar early changes are produced. These, however, do not heal but progress and form large abscesses containing amoebae. These lesions probably develop in infarcted areas, which include periportal lesions infected with amoebae and the associated bacteria.

The development of these persistent lesions is associated with the survival of amoebae in the affected area. We believe that this may result from «preparation» of the liver tissues for the reception of amoebae arising from the production of tissue sensitivity to antigens contained in the trophozoites.

We have demonstrated the development of such sensitivity by intradermal reactions, using an antigen obtained from the relevant strain of *E. histolytica* by freezing and thawing trophozoites, and using trypan blue as an indicator (Duthie and Chain, 1939; Menkin, 1936). Thus, in one experiment 25 guinea pigs were inoculated intracaecally with *E. histolytica* and 14 days later given intradermal injections of antigen made from the homologous strain. Of 17 which developed intestinal lesions, 13 gave strongly positive reactions; of the 8 with no lesions 4 gave positive reactions. In 8 normal controls

the reactions were all negative. Reactions to antigens made from associated bacteria were negative (Fig. 11).

We attempted in other experiments to «sensitize» guinea pigs with saline freeze-thaw extracts of amoebae and followed this procedure by subsequent portal inoculation of homologous amoebae. Sensitization was achieved, for example, in 33 animals injected with

Fig. 11 — Positive sensitivity reaction in skin following injection of *E. histolytica* antigen. (Guinea pig with chronic intestinal lesion)

3 successive doses of antigen. Sensitivity was demonstrated in the skin and liver substance of sample animals 3 weeks later. Intraportal inoculation of amoebae was performed in the remainder. The picture of the resulting hepatic changes was similar to that seen in «chronic intestinal» animals, and large macroscopic abscesses (with visible and culturable amoebae present) persisted up to the eighth day.

It seems possible therefore that the sensitization achieved influenced the establishment of the amoebae in the hepatic tissue of these animals.

It may be that the presence of the antigen (the trophozoites in this case) in the sensitized tissue may have led to antigen-antibody reactions which, in the liver, as we have shown elsewhere (Maegraith *et al.*, 1949), results in local circulatory disturbances which restrict and retard the local circulation of the blood and possibly help the formation of infarcts. This in turn may well lead to physiological conditions more suitable for the continued existence of the parasite and its multiplication in the affected tissue.

We believe reactions of this sort may be significant in other invasions of host cells and tissue by parasites. It is surely worth while finding out! (Maegraith, 1954*a*, *b*).

## BIBLIOGRAPHY


Anderson, H. H., Bostick, W. L., and Johnstone, H. G. — *Amoebiasis: pathology, diagnosis and chemotherapy*. Springfield, Ill. Charles C. Thomas, 1953.

Andrews, W. H. H., Maegraith, B. G., and Wenyon, C. E. M. — *Ann. trop. Med. Parasit.*, **43**: 229, 1949.

Andrews, W. H. H., Hecker, R., Maegraith, B. G., and Ritchie, H. D. — *J. Physiol.*, **122**: 51P, 1953.

Boyd, W. — *Textbook of Pathology*, 6th Ed. London, Kimpton, 1953.

Duthie, E. J., and Chain, E. — *Brit. J. exper. Path.*, **20**: 417, 1939.

Harinasuta, C., and Maegraith, B. G. — *Trans. Roy. Soc. Trop. Med. Hyg.*, **48**: 285, 1954.

Maegraith, B. G. — Ed. F. W. Hoffbauer. *Liver Injury*, Josiah Macy Foundation: 181, 1951.

Maegraith, B. G. — *Amer. Rev. Microbiol.*, **8**: 273, 1954*a*.

Maegraith, B. G. — *Proc. Roy. Soc. Med.*, **47**: 626, 1954*b*.

Maegraith, B. G. — *Mechanisms of Microbial Pathogenicity*. 5th Symposium, Society for General Microbiology, Cambridge University Press, pp. 207-229, 1955.

Maegraith, B. G., and Harinasuta, C. — *Trans. Roy. Soc. Trop. Med. Hyg.*, **47**: 582, 1953.

Maegraith, B. G., and Harinasuta, C. — *Trans. Roy. Soc. Trop. Med. Hyg.*, **48**: 421, 1954*a*.

MAEGRAITH, B. G., and HARINASUTA, C. — *Trans. Roy. Soc. Trop. Med. Hyg.*, **48**: 434, 1954*b*.

MENKIN, V. — *J. exper. Med.*, **64**: 485. 1936.

TAYLOR, D. J., GREENBERG, J., HIGHMAN, B., and COATNEY, G. R. — *Amer. J. trop. Med.*, **30**: 817, 1950.

TORPY, D. C., and MAEGRAITH, B. G. — *Trans. Roy. Soc. Trop. Med. Hyg.*, **51**: 1, 1956.

# PARASITOLOGIC SURVEYS IN CALI, DEPARTAMENTO DEL VALLE, COLOMBIA. III. ENVIRONMENTAL, NUTRITIONAL AND OTHER FACTORS RESPONSIBLE FOR THE HIGH INCIDENCE AND LOW PATHOGENICITY OF *ENTAMOEBA HISTOLYTICA* IN FAMILIES OF THE LOWER ECONOMIC CLASS IN CALI, COLOMBIA

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## INTRODUCTION

Special attention has been given to *Entamoeba histolytica* in connection with the parasitologic survey conducted among more than 1,000 persons belonging to 100 families and constituting a satisfactory sampling of an underprivileged ward (barrio Siloé) of the city of Cali, Colombia. Previously (Faust, 1958) the morphologic characters of the strain of this ameba in the Cali population have been described. The present report is concerned with the incidence and clinical significance of *E. histolytica,* and the relationship of environmental, nutritional and other factors to the infection. The goal of obtaining a minimum of 3 fresh fecal specimens from each individual in the families under study has met with appreciable success during the first year of the survey. Diagnosis was made by the authors of this

communication and competently trained technicians under their direct supervision. The technics employed were the standard direct fecal film examination in physiologic saline and iodine-stained preparations, supplemented by the zinc sulfate concentration method (Faust *et al.*, 1938, 1939).

## PRESENTATION OF DATA

A total of 1,714 fecal specimens from 837 persons were examined during the first year of study, with an average percentage incidence of 40.2 for *E. histolytica*. In only 19 individuals were all 3 or more stools positive for this ameba, while positive diagnosis was made twice from 63 persons and only once from 255 persons. Less than 3 examinations were performed on stools of 362 persons. By design the feces were secured at intervals of 1 to 3 months, in order to obtain evidence of maintenance or loss of the infections, or of newly acquired infections. These data are summarized in the table.

Although only 36.7 % of the population studied had provided 3 or more specimens at the end of the first year of study, there has been increasing evidence that infection with *E. histolytica* may be spontaneously lost and newly acquired over a period of several months to a year. If this conclusion is correct, it suggests two possible governing factors, namely (1) opportunities for exposure and reexposure must be relatively abundant, and (2) a majority of the infections may be only superficially established in the large intestine.

*Opportunities for exposure and reexposure.* — Epidemiologic studies, which have been conducted in this same population as part of the Family Care Program of the Department of Preventive Medicine and Public Health, University of Valle, have provided the following information. The climate of Cali is relatively warm throughout the year (20°C minimum and 26°C maximum), with two short rainy seasons, one in the fall and one in the spring. The families under investigation belong to the poorer, underprivileged class of the city of Cali. Their homes are situated on a mountain-side several kilometers from the center of the city. The streets are mostly rocky and rutted, winding up the hillside. The dwellings are brick-walled and tile-

roofed, but usually consist of only 2 or 3 rooms and are very crowded for the average family of 10 or more persons. Most of the houses have dirt floors. Water from the city aqueduct is available for the lower part of Siloé, but is ordinarily not piped to the homes. Latrines,

*Progress report on diagnosis of* Entamoeba histolytica *in families from ward Siloé, Cali, Colombia (Nov. 1956–Oct. 1957)*

| | | | % |
|---|---|---|---|
| 1. | Total persons in families studied | 1,085 | — |
| 2. | Total persons examined | 837 | 77.1 |
| | Once only | 306 | 28.1 |
| | 2 times | 224 | 20.7 |
| | 3 times | 268 | 24.7 |
| | 4 times | 39 | 3.6 |
| 3. | Total fecal specimens examined | 1,714 | — |
| | Average per person | 2 | — |
| 4. | Total persons positive for *E. histolytica* | 337 | 40.2 |
| | Positive each of 3 or more times | 19 | — |
| | Positive 2 times | 63 | — |
| | Positive from specimens 1 and 2 | 36 | — |
| | Positive from specimens 1 and 3 or 4 | 13 | — |
| | Positive from specimens 2 and 3 or 4 | 14 | — |
| | Positive only once | 255 | — |
| | Positive from specimen 1 | 173 | — |
| | Positive from specimen 2 | 49 | — |
| | Positive from specimen 3 | 29 | — |
| | Positive from specimen 4 | 4 | — |
| 5. | Total persons negative following 3 or 4 examinations | 138 | 16.5 |
| 6. | Persons with less than 3 examinations at end of study year | 362 | 43.3 |

when present, are very primitive and unsanitary; filth flies breed in the excreta and in the garbage and then swarm about the kitchen. Children play in the garbage, and hundreds of dogs, which roam everywhere in the area, consume both garbage and human excreta. Only one pig has been found in this district. Racially, the population consists of individuals of Spanish blood, mestizos, and mulattoes. Although for the most part uneducated, they have relatively high intelligence; yet very few families have any conception of personal hygiene or of the health hazards of an unsanitary environment.

Examination of the soil on the floors, in the courtyards and around the latrines has demonstrated that cysts of the human intestinal protozoa and eggs or larvae of human nematodes prevalent in the area are present in a viable condition in sufficient number to provide opportunity for repeated infection. Freshly passed excreta of dogs, deposited on the streets and in the courtyards throughout the ward when examined contained viable cysts of *Entamoeba histolytica* and other intestinal protozoa of man, as well as eggs of *Ascaris lumbricoides,* providing substantial evidence that human excreta are being continually consumed by the dogs and promiscuously disseminated. However, the most likely cause of the high prevalence of *E. histolytica* infection is person-to-person contact within the homes, due to almost complete lack of personal hygiene and environmental sanitation, with inadequate facilities for cleansing the hands, for bathing and for laundry purposes.

The average incidence of *E. histolytica* in Siloé is high (40 % on the basis of incomplete data). It reaches a level of nearly 50 % in the 5-to-9-year age group and maintains this figure through early adult life, then gradually declines. One positive diagnosis has been made in an infant 2 months of age and another in a 9-months-old child. The age distribution is graphically shown in Fig. 1. Moreover, amebiasis is much more prevalent in about a third of the families than in the population as a whole.

*Nutrition of Siloé families.* — With very few exceptions, the incomes of the Siloé families are submarginal so that a major part of expenditure is for food. This is by necessity primarily carbohydrate with a minimal amount of beef, fats and green vegetables. The principal foods are green plantains and yucca tubers, commonly cooked in a thick soup called *sancocho.* Most of the infants, when weaned, are placed principally on a diet of brown sugar water *(panela),* occasionally with a meager supplement of poor quality cow's milk for the youngest child. Families with slightly better incomes buy some maize, rice and beans, occasionally oranges and bananas, a little more beef, at times a little pork. The average child or adult consumes more starch than he is able to digest, due to pancreatic insufficiency, so that a considerable amount of undigested starch passes through the large intestine and is evacuated in the feces. While the nutritional deficiencies of the individuals in this population

have not as yet been carefully studied, the residuum of undigested starch *en transit* through the large intestine provides potential food for trophozoites of *Entamoeba histolytica* in the intestinal lumen, with the consequence that these amebae conceivably may grow and multiply without invasion of the intestinal wall. Four out of every

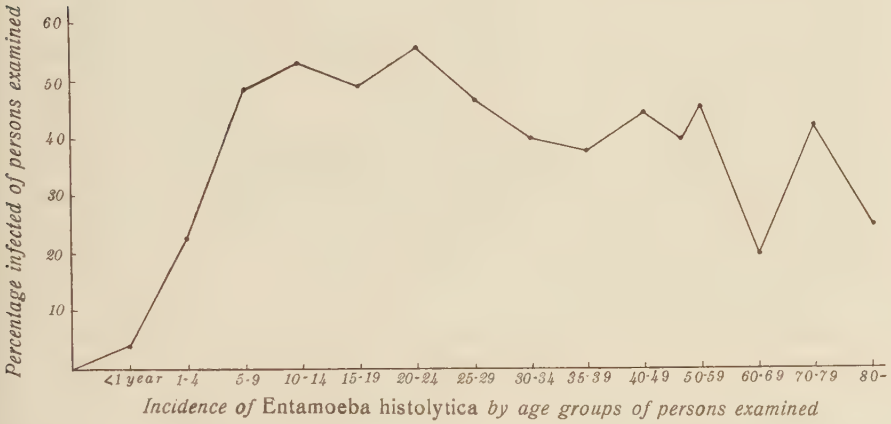


Fig. 1 — Incidence of *Entamoeba histolytica* by age groups in the 337 persons found infected of the 857 examined, based on 1,714 fecal examinations (ward Siloé, Cali, Colombia). In each age group the following numerators represent the number of positives, the denominators the number of individuals examined: less than 1 year, 2/55; 1-4 years, 36/160; 5-9 years, 94/194; 10-14 years; 63/119; 15-19 years, 30/71; 20-24 years, 20/36; 25-29 years, 21/45; 30-34 years, 12/30; 35-39 years, 18/48; 40/49 years, 25/56; 50-59 years, 10/22; 60/69 years, 2/10; 70-79 years, 3/7, and 80 years, or older, 1/4. The age groups in the sampling correspond to those for the total population of the section of the city of Cali in which the survey was conducted. The number of persons examined in the groups 60 years and older is too small for significance

5 strains of this species belong to the «large race» (Faust, 1958), which is considered to be highly pathogenic in tropical areas such as that in Cali, and to be the cause of amebic dysentery and amebic liver abscess.

However, physical examination of infected individuals, including proctoscopy, has failed to reveal clinical evidence of this infection except in two instances of amebic colitis, in which abundant trophozoites were evacuated in the diarrheic but non-dysenteric excreta.

Otherwise, the feces have been formed or semi-formed and have contained almost exclusively the cystic stage of the organism (Faust, 1958).

In order to explore the reasons for the high incidence and low rate of pathogenicity of *E. histolytica* among individuals in this population, two collateral studies were undertaken during the sumer of 1957. Ten strains of the large race of this ameba were cultured in standard monophasic and diphasic media. When luxuriant growth was obtained in culture, the organisms were introduced aseptically into cecum of young guinea pigs, in groups of 5 or more test animals for each strain, with replications in three instances. After 5 to 9 days the animals were sacrificed and examination was made of the cecum and adjacent colon. One or more animals of each series, and in some series all individuals, demonstrated macroscopic lesions typical of amebic origin, at times with diphtheritic membrane and deep colonization by the amebae, comparable to those described earlier by Carrera and Faust (1949). Microscopic examination of tissue sections has confirmed the macroscopic findings in 83 % of the animals. This study amply supports the thesis that the strains of the large race of *E. histolytica* in this population of Cali, Colombia, are intrinsically pathogenic, and under appropriate conditions are capable of invading the wall of the large intestine and producing colitis of severe clinical grade. Detailed report of this study is being published separately (Krupp and Faust, 1959).

The second study has concerned the ability of the large race of *E. histolytica* in this community to ingest and digest the types of starch commonly found in the fecal dejecta of the population. For this purpose the amebae were first cultured with rice starch, recognized as an excellent substrate for growth of this organism *in vitro*. When luxuriant growth had been obtained, transfers were made to tubes containing the following substituted starches: plantain, yucca, wheat, maize, potato, and white sweet potato, with rice starch as control. Fresh starch of the particular type was introduced into each subculture tube. Subinoculations were made every 48 hours for a period of 32 to 80 days, depending on the ability of the organisms to flourish on the particular starch diet. Although some rice starch was carried over in the earlier subcultures, by the fifth or sixth transplant microscopic examination indicated that only the starch being tested remained

for ingestion by the amebae. In many instances (wheat, potato and sweet potato) the starch grains ingested were as large as the ameba trophozoites (e.g., 25 $\mu$ to 35 $\mu$ or more in diameter). With the exception of maize, the amebae in culture appeared to be able to utilize the substituted starch for their metabolic needs as satisfactorily as rice starch. Cultures containing maize starch survived only to the fifteenth transfer. The other cultures were growing satisfactorily when the experiment was terminated at the end of 80 days. The data accumulated in this study indicate that the strains of *E. histolytica* tested are able to utilize the starches consumed by the population under study. When these carbohydrates are available *en transit* through the large intestine, it seems probable that this ameba can continue to thrive essentially as a lumen parasite. Details of this investigation are being published separately (Read and Faust, 1959).

## DISCUSSION

The high incidence of *Entamoeba histolytica* in the population of Siloé can be explained on the basis of the low grade personal hygiene and poor community sanitation, which provides repeated opportunity for ingestion of viable cysts as a result of person-to-person contact, from the ground on the premises and possibly from food contamined with feces. By ingestion of human feces, dogs also contribute to the dissemination of viable cysts of human origin evacuated in their excreta.

The presence of several varieties of undigested starch grains in the human large intestine, acceptable for the carbohydrate needs of these strains of *E. histolytica,* provides a tenable explanation for the overwhelming preponderance of non-clinical amebiasis in the population, since the amebae are presumably able to colonize in the intestinal crypts without, or with only superficial invasion of the intestinal wall. This type of host-parasite relationship may likewise provide a basis for the relatively frequent loss and reacquisition of the infection, as suggested by repeated failure to find the ameba on second or subsequent examinations following an original diagnosis, or discovery of the ameba only on second or subsequent examination.

Although severe amebic colitis and amebic liver abscess have not been encountered in the Siloé population, these complications are occasionally observed in the University Hospital in Cali, usually discovered on surgical intervention or at post-mortem.

The present investigation has focused attention on the high incidence of non-clinical amebiasis in a tropical community, with experimental proof of the potential high pathogenicity of the strains of *E. histolytica* involved. Because utilizable starch is available for the amebae in the lumen of the large intestine of the population under study, a tenable explanation is provided for colonization of these amebae in the intestinal lumen or superficially in the crypts, and for the frequent loss of the infection.

## SUMMARY AND CONCLUSIONS

1. In a parasitologic survey of 837 persons from 100 families in an economically poor section of Cali, Colombia, an average of 2 fecal specimens per person has revealed 40 % incidence of *Entamoeba histolytica* infection. The infection is acquired early in life, reaches its maximum between the years of adolescence and early adult life, then very gradually declines.

2. Due to low-grade personal hygiene and environmental sanitation, there is ample opportunity for repeated exposure. Fecal examinations suggest frequent spontaneous loss of the infection and reinfection.

3. The diet of the population is primarily carbohydrate, with very little supplements of animal proteins, fats and vitamins. The principal foods are green plantains and yucca tubers, less commonly potatoes, rice, wheat and maize. More starch is consumed than is digested, so that unmodified starch grains are commonly evacuated in the feces.

4. Although the strains of the large race of *E. histolytica* in the community under investigation are potentially highly pathogenic, as demonstrated experimentally in guinea pigs, clinical amebiasis is extremely rare. It is quite possible that the undigested starch grains of varieties acceptable to the ameba *en transit* through the large intestine provide the necessary metabolic requirements for this protozoan, so that it is able to live in the crypts of the large intestine in association with enterobacteria, and only rarely invades the intestinal wall deeply enough to produce permanent colonies and occasion clinical manifestations.

## BIBLIOGRAPHY

CARRERA, G. M., and FAUST, E. C. — «Susceptibility of the guinea pig to *Endamoeba histolytica* of human origin», *Am. J. Trop. Med.*, **29**: 647-667, 1949.

FAUST, E. C. — «Parasitologic surveys in Cali, Departamento del Valle, Colombia. I. Incidence and morphologic characteristics of strains of *Entamoeba histolytica*», *Am. J. Trop. Med. & Hyg.*, **7**: 4-15, 1958.

FAUST, E. C., D'ANTONI, J. S., ODOM, V., MILLER, M. J., PEREZ, C., SAWITZ, W., THOMEN, L. F., TOBIE, J., and WALKER, J. H. — «A critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in feces», *Am. J. Trop. Med.*, **28**: 169-183, 1938.

FAUST, E. C., SAWITZ, W., TOBIE, J., ODOM, V., PEREZ, C., and LINCICOME, D. R. — «Comparative efficiency of various technics for the diagnosis of protozoa and helminths in feces», *J. Parasitol.*, **25**: 241-262, 1939.

KRUPP, IRIS M., and FAUST, E. C. — «Parasitologic surveys in Cali, Departamento del Valle, Colombia. IV. Experimental study in guinea pigs of the pathogenic capacity of *Entamoeba histolytica* from infected persons in ward Siloé, Cali», *J. Parasitol.*, **45**: 449-455, 1959.

READ, T. R., and FAUST, E. C. — «Capacity of different strains of *Entamoeba histolytica* from infected persons in ward Siloé, Cali, Colombia, to ingest and digest starches consumed by the human hosts», *Am. J. Trop. Med. & Hyg.*, **8**: 293-300, 1959.

## ACKNOWLEDGEMENTS

We express our appreciation to Dr. Santiago Renjifo S., Chairman, Department of Preventive Medicine and Public Health, University of Valle, for providing field and laboratory facilities for this study; to Señorita Inés Mora, Chief Public Health Nurse of the Department, for assistance in obtaining the fecal specimens; to Dr. Jorge Lega, for proctoscopic examination of cases under investigation; to Señorita Gladys Caicedo, for diagnostic help, and to Doctor Iris M. Krupp and Mr. Thomas R. Read, of Tulane University, for expert technical assistance in the experimental studies.

## GENERAL DISCUSSION

Sir Philip Manson-Bahr (U.K.) — With reference to Dr. Beaver's paper on cellular exudates in the faeces, I would like to point out that I commenced the study of cellular exudate of the dysentery as long ago as 1910. Dr. J. G. Willmore wrote a classical paper on this subject in 1917 which was published in the *Lancet*. Anderson worked in my *tented* laboratory in Palestine and found Charcot-Leyden crystals and eosinophil cells in amoebic dysentery stools, mostly in Egyptian and Indian soldiers. We thought that there was a definite correlation between the eosinophil cells and Charcot-Leyden crystals, but it is dangerous to consider these crystals as diagnostic of amoebiasis for they occur with carcinoma and other forms of colitis. There is no doubt that in the cellular picture we have the means of differentiation from bacillary dysentery in which the chief elements are the polymorphonuclear and macrophage cells, the importance of which is that they were mistaken commonly for *Entamoeba histolytica* and that was the real tragedy of the great bacillary epidemic of Gallipoli in 1915.

Dr. Saul Adler (Israel) — In addition to intra-caecal inoculation it is useful to inoculate rabbits subcutaneously. With suitable strains subcutaneous amoebic abscesses are formed. These can be observed continuously without sacrificing the animals.

Dr. Brian G. Maegraith (U.K.) — With regard to Dr. Faust's statement I hope more work will be done on what the amoeba does to starch before we decide to connect the pathological effects of infection with the kind of starch present — the food, digested or otherwise. We have spent a couple of abortive years trying to find out with many kinds of starches and glycogen. I suppose we must wait until we can get bacteria free amoebae.

With regard to eosinophils, I suspect they are concerned with local antigen-antibody reactions which I think our work on liver lesions has shown to be important in amoebic pathogenic effects. Local effects in the bowel have been noted by others, for example, local immunity, and I suspect antigen-antibody reactions take place there too, with coincident tissue effects.

Dr. A. Neghme (Chile) — 22 years ago I had the opportunity to study the exudate of acute amebic dysenteric cases and I found 4 % to 6 % of eosinophils in the exudate, with or without Charcot-Leyden crystals. Incidentally, I should like to add that some of my collaborators in Santiago (Chile) have been able to find very regularly Charcot-Leyden crystals in human isosporosis.

Dr. E. Meerovitch (Canada) — Dr. Faust's observation that *E. histolytica* is largely non-pathogenic in natives of Cali, Colombia, due to the presence of undigested plant starches in their intestines is supported by our studies on the host-parasite relationship in reptile amoebiasis [1]. *E. invadens,* a strain of which was isolated by us from a turtle, was, as other strains, extremely pathogenic in snakes. The non-pathogenicity in turtles is regarded to be due to the adaptation of the parasite to the host, based on the vegetarian food habits of the turtles. Wholly carnivorous snakes could never have evolved a commensal relationship with *E. invadens.*

Prof. Woodruff (U.K.) — At the Hospital for Tropical Diseases, London, we have approximately 200 patients per annum suffering from intestinal infection with *Entamoeba histolytica.* Only a minority of these patients have coincidental helminthic or other infections and we have not noticed any special tendency for those with *E. histolytica* infections only, to have eosinophilia or an eosinophilic exudate in the stools. On the other hand, eosinophilia is commonly, even classically associated with helminthic infections. In Dr. Beaver's series there was only one patient who did not have an helminthic as well as an amoebic infection. It thus appears probable that the eosino-

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[1] *Can. J. Zool.,* **36**: 513-523, 1958.

philic exudates noted were caused by the presence of the helminths rather than by the amoebae.

Dr. Beaver's very interesting communication raises an important question. It must be very unusual to find a series of patients suffering from intestinal amoebic infections, 34 out of 35 of whom are also infected with either *Trichuris trichiura, Schistosoma mansoni* or *Strongyloides stercoralis* and if such a rate of infection with these parasites is representative of patients generally suffering from intestinal amoebiasis in Durban, to what extent is the severity of the disease, as it is seen there, due to coincident helminthic infection. These parasites are able to damage the mucous membrane of the large bowel and such traumatised areas would readily be invaded by *E. histolytica,* the reproduction of which would probably be facilitated by the presence of foci of necrotic or damaged cells. A heavy infection with much tissue damage would be expected then to develop and if the bowel is heavily infected, extension to other organs, as has been noted in Durban, would become probable. A mechanism of this kind need not necessarily be the only one responsible for the increased severity of amoebic infection, as seen in Durban. Perhaps Dr. Elsdon-Dew would give us his views on this matter.

3RD SESSION: **Clinical aspects of amoebiasis**

*Wednesday, September 10th — 9:00 to 10:30 a.m. Room C*

The session was opened under the chairmanship of Dr. C. Romaña and Dr. J. G. Basnuevo and Dr. R. Elsdon-Dew were the Rapporteurs. The Secretary was Dr. L. T. de Almeida Franco.

The papers presented and discussed in this session are the following.

# DIAGNÓSTICO Y TRATAMIENTO DEL ABSCESO HEPÁTICO AMIBIANO Y LAS ULCERACIONES INTESTINALES POR *ENDAMOEBA HISTOLYTICA*

JOSÉ G. BASNUEVO [1]
ENRIQUE FIGARES [2]
y
ESTEBAN GUTIÉRREZ ESTARLÍ [3]

## DIAGNÓSTICO Y TRATAMIENTO DEL ABSCESO HEPÁTICO AMIBIANO

Toda hepatomegalia febril y dolorosa, asociada a leucocitosis neutrófila en el hemograma, debe hacer pensar en absceso hepático amibiano, aunque en algunos casos de absceso hepático amibiano la

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hepatomegalia no es muy ostensible, la fiebre no es muy constante y la leucocitosis puede ser muy moderada.

La historia anterior de deposiciones de tipo disentérico será un dato más en favor de este diagnóstico, aunque dicha historia puede faltar por completo.

También debe ser tomado en consideración para pensar en absceso hepático amibiano todo proceso inflamatorio o congestivo de la base del pulmón derecho cuya etiología no esté muy clara.

La pleuresía purulenta del pulmón derecho algunas veces puede tener su origen en un absceso hepático amibiano que pasa inadvertido.

Sintomatología.

La fiebre de tipo remitente o intermitente puede ser de poca elevación o puede elevarse hasta 39,5°C; sudoración, escalofríos, anorexia, pérdida de peso, debilidad, malestar general, náuseas y vómitos, son bastantes frecuentes.

Existe dolor sordo y contínuo en la región hepática, trasmitido casi siempre al hombro derecho, con hipersensibilidad en todo el reborde costal del mismo lado.

Si el absceso crece hacia la superficie, puede notarse una tumefacción en el epigástrio, entre los espacios intercostales inferiores o en la región lumbar.

Por la percusión o palpación, o ambas cosas a la vez, se puede encontrar un aumento del área hepática, ya por encima, ya por debajo.

La palpación revela también algunas veces una ligera rigidez de la pared muscular en el hipocondrio derecho.

Si la pleura o el pulmón son invadidos, se nota disminución de la amplitud respiratoria, pudiendo auscultarse estertores en la base pulmonar derecha. La invasión pleural puede ser causa de disnea y tos sin expectoración.

La inspiración profunda puede dar lugar a dolor agudo. El dolor suele aliviarse al acostarse el paciente sobre el lado derecho.

Se ha señalado la frequencia del dolor a la compresión bimanual de la base del hemitórax derecho, que sería prácticamente constante y de valor diagnóstico diferencial, con otros procesos agudos de la región tóracoabdominal, con excepción de la colecistopatías agudas.

La ictericia no es frecuente, pero algunos presentan un tinte sub-ictérico en las conjuntivas. En los casos de larga evolución la piel puede presentar un tinte cetrino característico con facies especial de emaciación, apatía y cansancio.

En ocasiones existe historia anterior o actual de diarreas de tipo disentérico.

### Examenes complementarios.

En el hemograma encontramos leucocitosis (10.000 a 25.000) con polinucleosis (75 % a 90 %). La infección bacteriana eleva la cifra de leucocitos (15.000 a 35.000) con polinucleosis de 85 % a 95 %. En ocasiones los hematíes están disminuídos en número (2.500.00-4.500.000). Los eosinofilos en la fase aguda casi siempre están ausentes o disminuídos (0-2 %), muy pocas veces se encuentran ligeramente elevados (6-10 %). La eritrosedimentación se halla acelerada com cifras de 100 mm y más a la hora. El examen seriado de las heces fecales puede ser negativo de amibas.

La proctosigmoidoscopía puede mostrar lesiones ulcerativas intestinales. El raspado F2AM de la mucosa rectal es de gran utilidad para encontrar la *E. histolytica*.

Las pruebas funcionales hepáticas suelen ser normales o ligeramente alteradas, lo que es fácilmente explicable, dado que el absceso hepático es una hepatopatía focal y el resto del parénquima mantiene su integridad funcional.

### Examen radiológico.

Radiológicamente existe inmovilización del hemidiafragma derecho, que generalmente se encuentra elevado. La placa simple comprueba la hepatomegalia, y en los casos de absceso hepático que crece hacia la cúpula, abombando el diafragma, puede observarse una sombra redondeada haciendo prominencia en el campo pulmonar derecho (vista frontal y lateral).

El neumoperitoneo diagnóstico es de utilidad no sólo para afirmar la existencia del absceso, sino que nos ayuda en ocasiones a precisar su localización, ya que el hígado queda rodeado de una zona gaseosa que hace visible su contorno.

El examen radiológico nos proporciona también algunos detalles para hacer el diagnóstico diferencial con el absceso subfrénico. En el absceso hepático hay borramiento del ángulo cardiofrénico en la vista anteroposterior y del ángulo costofrénico anterior en la vista lateral, mientras que en el absceso subfrénico se encuentra obliterado el ángulo costofrénico en la vista anteroposterior y el ángulo costofrénico posterior en la vista lateral.

Si el absceso es del lóbulo izquierdo, pueden faltar estos signos radiológicos, pero si se hace ingerir bario al paciente, podremos observar que el cardias y la curvadura menor del estómago se encuentran desplazados hacia abajo, tomando esta última una forma de media luna. La invasión del lóbulo izquierdo del hígado es relativamente rara.

En caso de existir complicaciones pleuropulmonares, serán puestas en evidencia también por el examen radiológico.

### Visualización de los abscesos hepáticos.

Con alguna frecuencia los abscesos hepáticos pasan inadvertidos al examen radiológico simple del hígado. Pues el pus amibiano o bacteriano tiene poca diferencia con el parénquima hepático sano en lo que se refiere a la penetración por los rayos X.

La inyección de aire y lipiodol en la cavidad del absceso después de una punción evacuadora, realizando a continuación un estudio radiológico en distintas posiciones, nos mostrará el tamaño, los límites y la situación exacta de la cavidad del absceso. La hepatografía con el dióxido del torio no se ha generalizado.

### Diagnóstico positivo mediante la punción aspiradora.

Generalmente debe tomarse como punto de guía para la punción la parte del hígado más sensible y prominente.

Cuando no se comprueban signos de localización o de dolor bien limitados, lo mejor es puncionar en los 9.° y 10.° espacios intercostales, a nivel de la línea axilar media, dirigiendo la aguja hacia arriba y hacia la línea media en dirección al pezón, no introduciéndola a una distancia mayor de unos 7 cm a 9 cm de la pared toráxica. De esta manera, la aguja atraviesa la parte más importante del hígado y es

probable que alcance el absceso en la parte superior del lóbulo derecho, pues la mayor parte de los abscesos hepáticos se localizan en la parte posterior y superior del lóbulo derecho. La aguja o trócar utilizado debe ser de grueso calibre (n.º 15 o 18), pues casi siempre el pus amibiano es viscoso y espeso. Para hacer la punción bastará la infiltración local de piel y músculos con novocaína al 2 %.

Una vez en la cavidad del absceso, se aspirarán hasta uns 500 $cm^3$ del material contenido en el mismo, enviando al laboratorio muestras de las últimas cantidades aspiradas para exámenes parasitológico y bacteriológico. Las amibas son más frecuentes en las últimas cantidades aspiradas. Unos 2 $cm^3$ del material obtenido debe ser conservado en unos 10 $cm^3$ de la solución F2AM para exámenes posteriores.

A continuación debemos inyectar en la cavidad del absceso:

| | |
|---|---|
| Lipiodol tibio ... ... ... ... ... ... | 10 $cm^3$ |
| Aire ... ... ... ... ... ... ... ... ... | 50 $cm^3$ |

Tomándole después varias radiografías al enfermo en distintas posiciones, especialmente de pie, en anteroposterior y lateral, con el fin de obtener el tamaño, los límites y la situación exacta de la cavidad del absceso.

Cuando los abscesos son tratados clínicamente, el lipiodol en la cavidad del absceso facilita mucho las próximas punciones, haciendo más visible bajo pantalla fluoroscópica la cavidad del absceso, permitiendo al mismo tiempo seguir su evolución.

Cuando la punción hepática es positiva, las características del material extraído permiten, en general, *hacer una afirmación diagnóstica,* aunque en el examen microscópico no se observen las amibas.

El «*pus amibiano*» es un líquido puriforme bien ligado, de color achocolatado, espeso y viscoso, con estrías o pintas sanguinolentas algunas veces, constituído principalmente por detritus celulares, hematíes, algunos leucocitos y células hepáticas degeneradas. La contaminación bacteriana se caracteriza por un color amarillo o verdoso, con abundantes piocitos. Frecuentemente no se observan las amibas en el pus aspirado, pues ellas están situadas en las paredes de la cavidad del absceso. Nosotros las hemos encontrado solamente en el 25 % de nuestros casos.

TRATAMIENTO.

En el tratamiento del absceso hepático amibiano, la aplicación local, en la cavidad del absceso, de un buen amibicida no debe ser cuestión secundaria. Lo mismo sucede con la aplicación local de algunos antibióticos tales como la penicilina y la estreptomicina.

Después de cada aspiración, que debe ser lo más abundante posible, debemos dejar en la cavidad del absceso unos 25 $cm^3$ de la siguiente solución:

| | |
|---|---|
| Cloroquina al 5 % ... ... ... ... | 5 $cm^3$ |
| Penicilina ... ... ... ... ... | 500.000 unid. |
| Estreptomicina ... ... ... ... ... | 0,50 g |
| Suero fisiológico ... ... ... ... | 20 $cm^3$ |

Varias punciones y aspiraciones bien dirigidas, y el uso adecuado de una solución de difosfato de cloroquina al 5 %, dejada en la cavidad del absceso después de cada aspiración, disminuyen notablemente la posibilidad de la intervención quirúrgica en el tratamiento del absceso hepático amibiano.

Cloroquina por vía oral, mezclada en la porporción de 1:3 con diyodohidroxiquinoleína, debe ser administrada al mismo tiempo.

| | |
|---|---|
| Difosfato de cloroquina ... ... ... | 0,10 g |
| Diyodohidroxiquinoleína ... ... ... | 0,30 g |

*Adultos:* Difosfato de cloroquina, 0,50 g al día, durante 20 días. Diyodohidroxiquinoleína, 1.50 g al día, durante 20 días.

*Niños:* Difosfato de cloroquina, 0,01 g por libra de peso, hasta las 50 libras, al día, durante 20 días. Diyodohidroxiquinoleína, 0,03 g por libra de peso, hasta las 50 libras, al día, durante 20 días.

Aunque generalmente la caída de la fiebre se produce en los tres primeros días después de realizada la primera punción y haber tomado las primeras dosis de la mezcla cloroquina-diyodohidroxiquinoleína, la penicilina y la estreptomicina por vía intramuscular a la dosis de 500.000 unidades de penicilina y 0,50 g de estreptomicina cada 12 horas, durante unos 10 días consecutivos, será muy útil para combatir o prevenir una infección bacteriana secundaria.

*Cloroquina y emetina intramuscular.*

Como tratamiento coadyuvante se pueden administrar por vía intramuscular pequeñas cantidades de cloroquina y emetina.

Difosfato de cloroquina, 0,01 g por cada 10 libras de peso, al día, durante 20 días.

Clorhidrato de emetina, 0,001 g por cada 10 libras de peso, al día, durante 20 días (no es necesario inyectar una dosis superior a 0,01 g de emetina al día).

Ambos medicamentos pueden ser inyectados al mismo tiempo, utilizando una solución mixta de cloroquina al 5 % y emetina al 0,5 %.

Aunque es posible que el paciente abandone el hospital entre los 25 y 45 días de comenzado este tratamiento, es necesario realizar reconocimientos bimensuales durante unos seis meses aproximadamente.

Los examenes radiográficos seriados, el estado general del enfermo, así como el hemograma y la eritrosedimentación, serán muy bien valorados para dar como curado al paciente.

Generalmente frente a un caso de absceso hepático amibiano nosotros aplicamos el siguiente tratamiento:

Cloroquina-diyodohidroxiquinoleína por vía oral.

Penicilina-estreptomicina intramuscular y en la cavidad del absceso. Punción y aspiración del absceso cada tercer o quinto día, dejando después de cada punción, en la cavidad del absceso, 5 $cm^3$ a 10 $cm^3$ de una solución de cloroquina al 5 %.

Suero glucosado y transfusiones de sangre o de plasma cada vez que se estimen necesarios. Complejo B. Vitaminas $B_1$ y $B_{12}$. Extracto hepático. Betaina. Colina. Metionina. Inositol. Gluconato de calcio. Ácido fólico. Aminoácidos. Vitaminas y minerales. Gluconato de hierro y de cobalto. Levaduras, etc. Dieta de fácil digestión, dándole preferencia a los prótidos e hidratos de carbono.

En la primera semana después de comenzado este tratamiento, decidimos si el paciente debe ser operado. Generalmente, en este tiempo desaparece toda o casi toda la sintomatología; entonces, los exámenes radiográficos seriados, con el hemograma y la eritrosedimentación darán la pauta a seguir.

Si el hemograma y la eritrosedimentación tienden a ser normales, el hígado disminuye de tamaño, la cavidad del absceso se hace más

pequeña, y el paciente aumenta de peso a razón de unas 4 onzas por día, el drenaje abierto es innecesario y el enfermo va hacia la curación total y definitiva.

### Las punciones repetidas y la operación abierta en el tratamiento del absceso hepático amibiano.

Desde hace mucho tiempo se discute si el absceso hepático amibiano debe ser drenado por el método de las punciones repetidas, o por la operación abierta *(open operation)*.

Nosotros preferimos, siempre que sea posible, el método de las punciones repetidas, dejando después de cada aspiración en la cavidad del absceso unos 5 $cm^3$ a 10 $cm^3$ de una solución de cloroquina al 5 % adicionada de penicilina y estreptomicina. Aún en los grandes abscesos, contaminados o no contaminados, el método de las punciones repetidas nos ha proporcionado muy buenos resultados. La operación abierta, si es necesario realizarla, debe ser discutida. Actualmente, en el Hospital Universitario General Calixto García la mortalidad por absceso hepático amibiano es casi nula.

### *Cloroquina y emetina en el tratamiento del absceso hepático amibiano.*

Cloroquina es mejor que emetina en el tratamiento del absceso hepático amibiano. Frente a un caso de hepatitis amibiana o absceso hepático amibiano, cloroquina es la droga de elección. Pero como ambos medicamentos no se excluyen, un tratamiento combinado a base de cloroquina y emetina puede ser perfectamente realizado. Aunque generalmente la emetina se administra a la dosis de 0,01 g por cada 20 libras de peso, al día, durante 10 a 15 días, cuando la utilizamos combinada con la cloroquina no inyectamos una dosis superior a 0,01 g al día, durante 20 días (0,20 g como dosis total).

La oxitetracilina y la clorotetracilina son ineficaces contra la *E. histolytica* en su localización hepática. En 1955 Gordon, Wilmot y Elsdon-Dew reportaron 5 casos de absceso hepático amibiano curados con camoquín.

## DIAGNÓSTICO Y TRATAMIENTO DE LAS ULCERACIONES INTESTINALES POR *ENDAMOEBA HISTOLYTICA*

La proctosigmoidoscopía es el mejor procedimiento para el diagnóstico de las ulceraciones intestinales por *Endamoeba histolytica*. El examen microscópico del raspado de la mucosa rectal o de las ulceraciones complementa el examen rectosigmoidoscópico y eleva su importancia como método de diagnóstico. La solución F2AM actuando como método de concentración, fijador y colorante aumenta las posibilidades de encontrar la *E. histolytica*.

*Proctosigmoidoscopía.*

Frecuentemente se observan elevaciones papulares amarillentas, rodeadas de un halo rojizo, llenas de un líquido viscoso, que se comunican con la submucosa. Al examen microscópico del contenido de estas elevaciones papulares, se observan a veces numerosos trofozoítos de *E. histolytica* con hematíes en su protoplasma.

Otras veces se observan ulceraciones pequeñas, que pueden ser casi puntiformes y muy superficiales, que corresponden a lesiones de comienzo.

Posteriormente, y debido a la rápida proliferación de este protozoario, se forman grandes nidos de amibas, que destruyen los tejidos a su alrededor, formando úlceras profundas de bordes elevados rodeadas de zonas hemorrágicas. La mucosa situada entre estas ulceraciones casi siempre es de color y aspectos normales.

Cuando las lesiones submucosas se extienden y se ha coleccionado en ellas mucho «pus amibiano» [1], éste se abre paso hacia la luz intestinal, dando lugar a una úlcera de bordes socavados, cuello estrecho y base más ancha *(úlcera en forma de botella)*. Otras veces, estas lesiones se extienden en superficie, en dirección horizontal, tomando la forma de un «*botón de camisa*». Estas lesiones pueden comunicarse unas con otras por debajo de la mucosa sana, formando verdaderos túneles, conteniendo tejidos citolizados, donde las amibas se multi-

[1] Líquido puriforme, formado principalmente por mucus, hematíes, detritus celulares y trofozoítos de *E. histolytica*.

plican rápidamente y encuentran un medio favorable para su desarrollo y alimentación, formado principalmente por hematíes, leucocitos y restos de células tisulares.

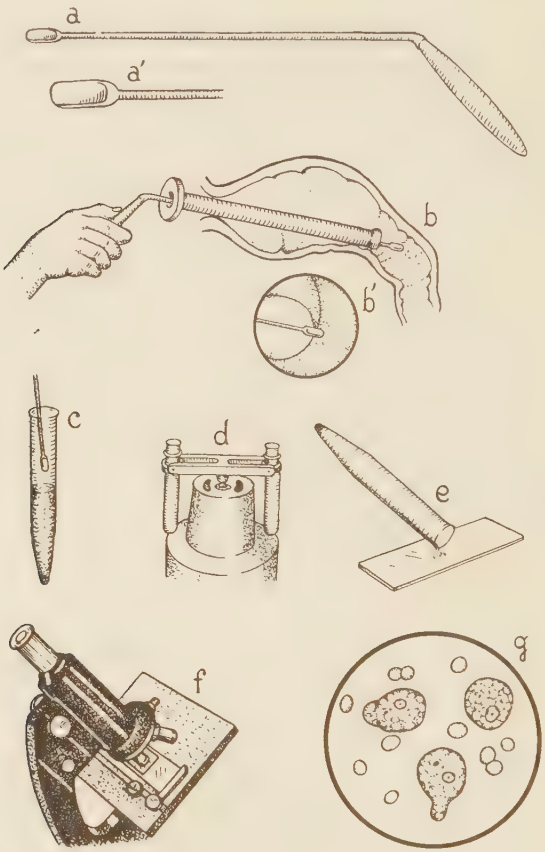


Fig. 1 — Raspado F2AM de la mucosa rectal para el diagnóstico de la amibiasis (según Basnuevo y Figares): *a*, *a'*, cucharrilla de mango largo; *b*, *b'*, como hacer el raspado de la mucosa rectosigmoidea; *c*, tubo de centrífuga conteniendo unos 3 cm³ de la solución F2AM para recoger el material procedente de los raspados; *d*, antes del examen, centrifugar durante unos 3 minutos a razón de unas 2,000 revoluciones por minuto; *e*, verter el líquido que sobrenada y colocar una gota del sedimento sobre un portaobjetos; *f*, cubrir con un cubreobjetos bien fino y examinar al microscopio utilizando lentes de pequeño y gran aumento; *g*, las amibas aparecen coloreadas de azul claro. Los hematíes, muy bien conservados, se presentan de color amarillo-verde muy claro

El tamaño y número de las ulceraciones es muy variable; pueden ser pequeñas y abundantes, o grandes y escasas, o viceversa, llegando algunas veces hasta la capa muscular del intestino, y aún producir perforaciones intestinales.

La colitis ulcerosa idiopática y la disentería bacilar enmascaran algunas veces estas típicas lesiones producidas por la *E. histolytica* en su localización intestinal.

*Raspado de la mucosa rectal o de las ulceraciones.*

Antes del examen rectosigmoidoscópico los pacientes recibirán dos enemas de agua tibia salada al 1 %. Uno la noche anterior y otro a la mañana siguiente, dos horas antes del examen.

Con una cureta similar a la de Worlkmann con el mango largo, que se emplea en las operaciones de las vías biliares, se recoge primero un poco del mucus o secresión que puede recubrir en ese momento la mucosa o las ulceraciones, después se raspa delicadamente la mucosa rectal en los sitios donde aparezca más alterada.

El material obtenido debe ser colocado en un tubo de centrífuga conteniendo unos 5 $cm^3$ de la solución F2AM lista para ser usada.

En el momento del examen el líquido debe ser centrifugado y el sedimento formado examinado al microscopio con lentes de pequeño y gran aumento. Los trofozoítos y los quistes de la *E. histolytica* aparecen coloreados de azul claro y se conservan en buenas condiciones durante unos dos meses aproximadamente. Cuando se estime necesario se puede recurrir al lugol o a la hematoxilina.

La *E. histolytica* debe ser investigada también en todo proceso ulcerativo o fistuloso de la región anal o perianal. Desde 1933 Kourí y colaboradores han demostrado de manera fehaciente que algunos casos de ulceraciones extensas y profundas de la región perianal, simulando un proceso neoplásico, pueden ser de origen amibiano. Las amibas se han observado en el pus o en la biopsia realizada.

SOLUCIÓN F2AM

A) Solución Stock (S-F2AM):

| | |
|---|---|
| Azul de metileno ... ... ... ... ... ... ... ... | 2 g |
| Alcohol de 95° ... ... ... ... ... ... ... ... ... | 40 $cm^3$ |

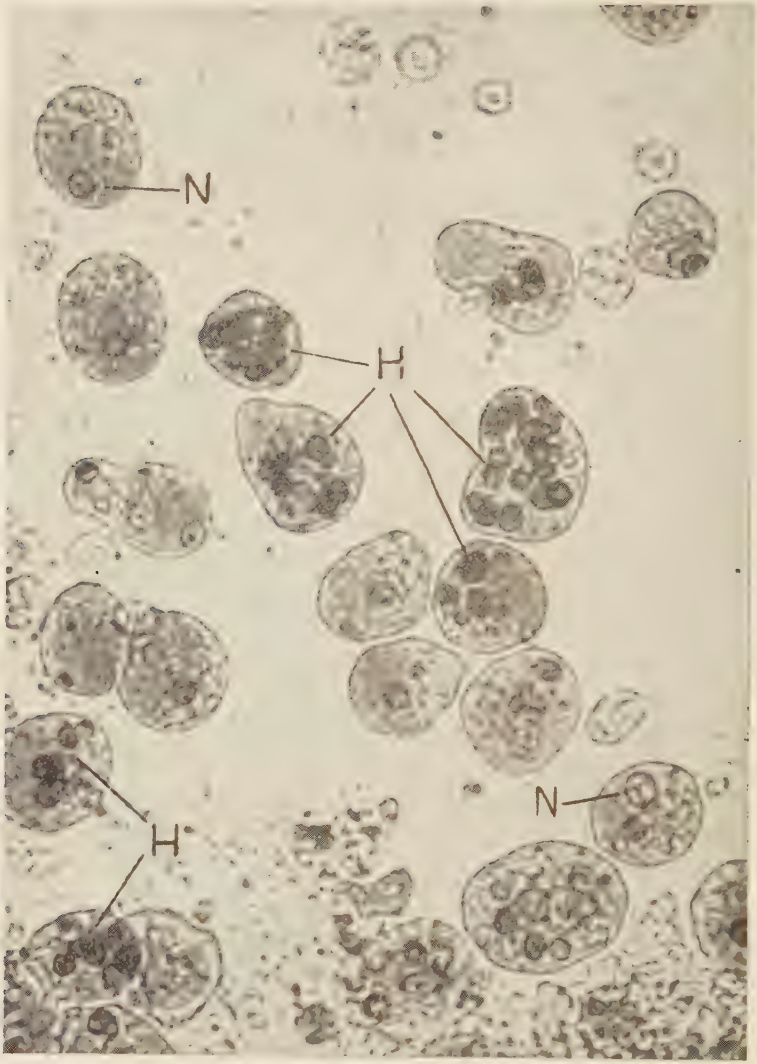


Fig. 2 — Raspado F2AM de la mucosa rectal. 20 trofozoitos de *Endamoeba histolytica* obtenidos por raspado F2AM de la mucosa rectal. La microfotografía fué realizada 12 días después de obtenida la muestra y conservada a la temperatura ambiente en la solución F2AM (ocular 10; objetivo 20). Los trofozoitos aparecen coloreados de azul claro, sobre un fondo azul más claro todavía. En algunos de ellos (N), la estructura nuclear se destaca notablemente. En otros, los hematíes (H) englobados en el protoplasma son característicos. En la solución F2AM los trofozoitos de la *Endamoeba histolytica* se conservan en buenas condiciones por más de 2 meses. La solución F2AM sustituye ventajosamente a los métodos con la hematoxilina férrica en el diagnóstico rápido y sencillo de la amibiasis. Cuando los examenes fecales son negativos, el raspado F2AM de la mucosa rectal es el método de elección en el diagnóstico de la amibiasis intestinal

| | |
|---|---|
| Acido fénico ... ... ... ... ... ... ... ... ... ... | 8 cm³ |
| Agua ... ... ... ... ... ... ... ... ... ... ... ... | 40 cm³ |
| Formol (40 %) ... ... ... ... ... ... ... ... ... ... | 10 cm³ |

B) Solución F2AM lista para ser usada:

| | |
|---|---|
| Solución Stock (S-F2AM) ... ... ... ... ... ... | 1 cm³ |
| Acido fénico ... ... ... ... ... ... ... ... ... ... | 0,10 cm³ |
| Agua ... ... ... ... ... ... ... ... ... ... ... ... | 89 cm³ |
| Formol (40 %) ... ... ... ... ... ... ... ... ... ... | 10 cm³ |

Ambas soluciones, colocadas separadamente en frasco de cristal, se conservan en buenas condiciones durante 5 años.

Tratamiento.

1) *Vía oral.*

*a*) Difosfato de cloroquina: 0,01 g por libra de peso, hasta las 50 libras, al día, durante 20 días.

*b*) Diyodohidroxiquinoleína: 0,03 g por libra de peso, hasta las 50 libras, al día, durante 20 días. Después de las 50 libras de peso no es necesario administrar una dosis superior a 0,50 g de cloroquina y 1,50 g de diyodohidroxiquinoleína, al día.

Se prefiere administrar ambos medicamentos conjuntamente, asociándolos en la proporción de 1:3, conteniendo cada tableta 0,10 g de difosfato de cloroquina y 0,30 g de diyodohidroxiquinoleína. Tomar las tabletas repartidas en el día.

2) *Vía rectal.*

Enemas de cloroquina, 1 g. Diyodohidroxiquinoleína, 3 g. Ftalilsulfacetamida, 4 g. Goma tragacanto, 3 g. Kaolín coloidal, 19 g. Agua tibia hervida, 1.000 cm³.

Volumen de los enemas: adultos, 1.000 cm³ a 1.500 cm³. Niños, 100 cm³ a 150 cm³ por año de edad aparente (15 cm³ a 20 cm³ por libra de peso). Hacer un enema cada tercer día hasta hacer 6 enemas en total. Dos horas antes del enema medicamentoso hacer un enema de limpieza con agua tibia salada al 1 %.

3) *Vía intramuscular.*

Como terapéutica auxiliar al tratamiento antes expuesto:

*a*) Difosfato de cloroquina: 0,01 g por cada 10 libras de peso, al día, durante 20 días.

*b*) Clorhidrato de emetina: 0,001 g por cada 10 libras de peso, al día, durante 20 días. (No es necesario inyectar una dosis superior a 0,01 g de emetina al día.)

Cloroquina y emetina pueden ser inyectadas al mismo tiempo, utilizando una solución mixta de difosfato de cloroquina al 5 % y clorhidrato de emetina al 0,5 %.

Además, si se estima necesario, penicilina y estreptomicina intramuscular. Ftalilsulfacetamida y neomicina oral. Terramicina. Aureomicina, etc.

Generalmente la curación, tanto clínica como parasitológica, se obtiene con la mezcla cloroquina-diyodohidroxiquinoleína por vía oral y los enemas de cloroquina-diyodo-ftalilsulfacetamida, no siendo necesario en la mayor parte de los casos recurrir a otros amibicidas.

Cuando utilizamos oxitetracilina (terramicina) o clorotetracilina (aureomicina), administramos al mismo tiempo cloroquina, a la dosis expuesta anteriormente.

Por su relativa toxicidad, los arsenicales y la emetina han pasado a ocupar un segundo plano en el tratamiento de la amibiasis. No tenemos experiencia personal con la conessine y el yoduro de bismuto y emetina, cuyo empleo no se ha generalizado en América.

Elsdon-Dew y Frye han obtenido muy buenos resultados con la oxitetracilina y otros antibióticos en el tratamiento de las ulceraciones intestinales por *E. histolytica.*

## RESUMEN

Toda hepatomegalia febril y dolorosa, asociada a leucocitosis neutrófila en el hemograma, debe hacer pensar en absceso hepático amibiano, aunque en algunos casos de absceso hepático amibiano la hepatomegalia no es muy ostensible, la fiebre no es muy constante y la leucocitosis puede ser muy moderada.

Los examenes radiográficos, la eritrosedimentación y la punción hepática ayudarán mucho para un buen diagnóstico.

Cuando la punción hepática es positiva, las características del material extraído permiten, en general, hacer una afirmación diagnóstica, aunque en el examen microscópio no se observan las amibas.

También debe ser tomado en consideración para pensar en absceso hepático amibiano todo proceso inflamatorio o congestivo de la base del pulmón derecho cuya etiología no esté muy clara.

El diagnóstico de las ulceraciones intestinales por *Endamoeba histolytica* se basa en el examen rectosigmoidoscópico y en el raspado F2AM de la mucosa rectal o de las ulceraciones.

La *E. histolytica* debe ser investigada también en todo proceso ulcerativo o fistuloso de la región anal o perianal.

En el tratamiento del absceso hepático amibiano cloroquina es mejor que emetina.

Administrando por vía oral una mezcla de cloroquina y diyodohidroxiquinoleína (1:3), e instilando en la cavidad del absceso, después de cada aspiración, una solución al 5 % de cloroquina, casi nunca es necesario recurrir a otros amibicidas. Siempre que sea posible se debe preferir el método de las punciones repetidas. Como terapéutica auxiliar se pueden administrar por vía intramuscular pequeñas cantidades de cloroquina y emetina. Para combatir la infección bacteriana secundaria preferimos la penicilina y la estreptomicina por vía intramuscular y en la cavidad del absceso.

Para el tratamento de las ulceraciones intestinales por *E. histolytica* preferimos la mezcla cloroquina-diyodohidroxiquinoleína (1:3) administrada por vía oral y rectal en enemas. La emetina y los arsenicales, por su relativa toxicidad, han pasado a ocupar un segundo plano en el tratamiento de la amibiasis. Los antibióticos y las sulfas pueden ser utilizados cuando se estimen necesarios.

## SUMMARY

Any feverish and painful hepatomegalias, associated to leukocytosis neutrophilous in blood cell count, must make think of amoebic liver abscess, although in some cases hepatomegalia is not very ostensible, fever is not constant and leukocytosis may be moderate.

The radiographic investigations, sedimentation rate, and hepatic punctures will help to establish a correct diagnosis.

When the hepatic puncture is positive, the characteristics of the material obtained lead to an affirmative diagnosis although no amebas are observed at the microscopic test.

Also must be taken as evidence, in considering an amoebic liver abscess, any inflammatory or congestive process on the base of the right lung, which etiology is not very clear.

The diagnosis of intestinal ulcerations by *Endamoeba histolytica* is based upon the rectosigmoidoscopic examination and F2AM scrapping of the rectal mucosa or of the ulcerations.

The *E. histolytica* must be investigated also in any ulcerative or fistulous process on the anal and perianal regions. In the treatment of amoebic liver abscess chloroquine is better than emetine.

Giving chloroquine-diyodohidroxiquinoline (1:3) orally, and instilling in the cavity of the abscess, after each puncture, a 5 % chloroquine solution, is usually enough and it is not necessary to apply to other amebicides.

The repeated punctures method is the best way to follow, whenever it is possible, in cases of amoebic liver abscess. Little amounts of chloroquine-emetine intramuscularly may be administered as an auxilliary therapy. To attack secondary bacterial infection we prefer penicillin and streptomycin intramuscularly and in the cavity of the abscess.

For the treatment of intestinal ulcerations by *E. histolytica* we prefer chloroquine-diyodohidroxiquinoline (1:3) orally, and also by rectal enema.

Emetine and arsenicals, because of its relative toxicity, have come to occupy a second place in the treatment of amoebiasis. Antibiotics and sulfas may be used when they are considered necessary.

## BIBLIOGRAFÍA


1. Basnuevo, J. — «Cloroquina y emetina intramuscular en el tratamiento de la amibiasis, la fascioliasis y la clonorchiasis», *Rev. Kuba Med. Trop.*, **11** (1-6): 5, 1955.
2. Basnuevo, J., Cowley, O., y Blanco-Rabassa, E. — «La cloroquina (tanakán) en el tratamiento de las ulceraciones intestinales por *Endamoeba histolytica* (II)», *Rev. Kuba Med. Trop.*, **7** (11-12): 143, 1951.
3. Basnuevo, J., Dominguez Quesada, S., y García, Mauri Clotilde — «Absceso hepático amibiano curado con cloroquina (baskir compuesto). — Anteriormente tratado sin éxito con terramicina», *Rev. Kuba Med. Trop.*, **8** (1-3): 6, 1952.
4. Basnuevo, J., y Figares, E. — «La solución F2AM y el raspado de la mucosa rectal en el diagnóstico de la amibiasis», *Rev. Kuba Med. Trop.*, **13** (7-12): 1957.
5. Basnuevo, J., Guerra Valdés, R., Gutiérrez Estarlí, E., y Sánches Beltrán, O. — «Absceso hepático amibiano complicado (pulmón) curado con tanakán (cloroquina)», *Rev. Kuba Med. Trop.*, **6** (3-4): 33, 1950.
6. Basnuevo, J., y Gutiérrez Estarlí, E. — «Absceso hepático amibiano curado con cloroquina», *Rev. Kuba Med. Trop.*, **5** (3-4): 31, 1949.
7. Basnuevo, J., y Gutiérrez Estarlí, E. — «Cloroquina y absceso hepático amibiano. Reporte de seis casos curados con cloroquina (tanakán-diyodohidroxiquinoleína)», *Rev. Kuba Med. Trop.*, **5** (9-10): 133, 1949.
8. Basnuevo, J., y Gutiérrez Estarlí, E. — «Nuevas orientaciones en el tratamiento del absceso hepático amibiano», *Rev. Kuba Med. Trop.*, **6** (9-10): 135, 1950.

9. Basnuevo, J., Gutiérrez Estarlí, E., y cols. — «Solución de cloroquina y bacitracina para aplicación local en el tratamiento del absceso hepático amibiano», *Rev. Kuba Med. Trop.*, **7** (3-4): 29, 1951.
10. Basnuevo, J., Miyares Ibarra, C., y Seuc Chiu, A. — «Absceso hepático amibiano con pleuresía purulenta curado con cloroquina y bacitracina», *Rev. Kuba Med. Trop.*, **6** (11-12): 157, 1950.
11. Basnuevo, J., Pulido Pérez, J. F., y Marrero Taboada, E. — «Absceso hepático tratado con aureomicina y cloroquina (baskir)», *Rev. Kuba Med. Trop.*, **8** (10 y 12): 81, 1952.
12. Basnuevo, J., y Vázquez Rosales, G. — «Un nuevo caso de absceso hepático amibiano curado con cloroquina (por vía oral y en instilación local en la cavidad del absceso)», *Rev. Kuba Med. Trop.*, **5** (11-12): 149, 1949.
13. Basnuevo, J., y Vázquez Rosales, G. — Absceso hepático amibiano perforado curado con emetina y cloroquina», *Rev. Kuba Med. Trop.*, **6** (3-4): 54, 1950.
14. Basnuevo, J. — «Diagnóstico de certeza del parasitismo intestinal en Cuba. Solución F2AM. Material obtenido por rectoscopía», *Rev. Kuba Med. Trop.*, **11** (7-12): 53, 1955.
15. Blanc, F., et Signier, F. — «Le traitement d'entretien de l'amibiase intestinale», *Gazette Médicale de France*, p. 817, fasc. 1, t. 57, n.° 16, 1950.
16. Centurión, J., Ballesteros, E., García Palacio, A., Douglas, R., y Cortés, J. — «Reporte de tres casos de absceso hepático amibiano tratados con cloroquina, con fracaso de terramicina», *Rev. Kuba Med. Trop.*, **10** (1-6): 1, 1954.
17. Conan, N. J. — «Chloroquine in amebiasis», *The American Journal of Tropical Medicine*, **28** (1), 1948.
18. Elsdon-Dew, R. — *Amoebiasis. A summary of literature published in 1953.* Printed by Pioneer Press. Forgate Street Woodstock. Cape.
19. Elsdon-Dew, R. — *Antibiotics in Acute Ulcerative Amebic Colitis,* reprinted from *Antibiotics Annual Medical Encyclopedia Inc.,* New York, N.Y., U.S.A., 1954-1955.
20. Elsdon-Dew, R. — *Amoebiasis in Africans,* reprinted from *Central African Journal of Medicine*, **2** (8): 291, 1956.
21. Faust and Russell — *Clinical Parasitology.* Sixth edition. Lea and Febiger. Philadelphia, U.S.A., 1957.
22. Fernández de la Arena María — «El absceso hepático amibiano en el lactante». Tesis para el Doctorado en Medicina. (Instituto de Medicina Tropical.) *Archivos Med. Infantil*, **25** (1): 1, Habana, Cuba, 1956.
23. Frye, W. W. — «The pathogenesis and therapy of human amebiasis», *American Journal of Gastroenterology*, **25** (4): 315, 1956.
24. Gordon, G., Wilmot, A., and Elsdon-Dew, R. — «Camoquín in amoebic liver abscess», *The Journal of Trop. Med. and Hyg.*, **58** (6): 133, 1955.
25. Kredi, E., Pire, A., y Pardo, G., — «Consideraciones clínicas sobre tres casos de absceso hepático», *Rev. Kuba Med. Trop.*, **10** (7-12): 56, 1954.

26. Kourí, P., Basnuevo, J., Llanio, E., y Sotolongo, F. — *Lecciones de Parasitología y Med. Tropical*, t. III: *Protozoología Médica*. Habana, Cuba, 1947.
27. Kourí, P., y Basnuevo, J. — «Amibiasis recto-ano-cutáneo-perineal. Causas de error en su diagnóstico. Conducta a seguir en su tratamiento», *Rev. Kuba Med. Trop.*, **11** (7-12): 42, 1955.
28. Kourí, P., Iriondo, M., y Fusté, R. — «Amibiasis cutánea por *Endamoeba histolytica*», *Rev. Kuba Med. Trop.*, **3** (4): 98, 1947.
29. Macías Castro, I., y Bencomo Martín, L. — «Recto-colitis ulcerosa de origen amibiano tratada con la asociación cloroquina-diyodohidroxiquinoleína por vía oral y en enemas simultáneamente», *Rev. Kuba Med. Trop.*, **9** (1-6): 1, 1953.
30. Manson-Bahr, P. — *Disenteric Disorders*. Second edition. The Williams-Wilkins Comp. Baltimore. Maryland. U.S.A., 1945.
31 Manson-Bahr, P. — «The treatment of amoebic liver abscess with chloroquine», *Tropical Medicine and Hygiene*, May, England, 1949.
32. Manson-Bahr, P., and Muggleton, W. — «Rectal biopsy as an aid to the diagnosis of amoebic dysentery and allied diseases of the colon», *The Lancet*, p. 763, April 13, 1957.
33. Martín Jiménez, R., Sosa Bens, S., Borges, F., y Varona, E. — «Absceso hepático amibiano en un lactante», *Archivos Med. Infantil*, **25** (2): 122, Habana, Cuba, 1956.
34. Moreno, O., Valdés Gutiérrez, O., Perea, J., y Casanova, R. — «Absceso hepático amibiano múltiple en el lactante. Reporte de un caso curado», *Revista Cubana de Pediatria*, **28** (10): 597, 1956.
35. Scaffidi, V., y Volsi, M. L. — «Sulla terapia delle epatiti colliquative amebiche con clorochina», *Acta Médica Italica di Malattie Infettive e Parassitarie*, **9**: 1, 1954.
36. Stapler, N. — «Diagnóstico de la amibiasis intestinal por el raspado de la mucosa rectal», *La Prensa Médica Argentina*, **38** (27): 1508, 1950.
37. Sodeman, W. A., *et al.* — «Chloroquine in hepatic amebiasis», *Annals of Internal Medicine*, **35** (2): 331, U.S.A., 1951.
38. Spicknall, Ch., and Bozicevich, J. — «The complement fixation test in the diagnosis of amebiasis», *Gastroenterology*, **32** (6), U.S.A., 1957.
39. Terry, L., Bozicevich, J., *et al.* — «The importance of the complement fixation test in amebic hepatitis and liver abscess», *Southern Medical Journal*, **41** (8): 691, Alabama, U.S.A., 1948.
40. Velazco, R., Amado Ledo, E., y Gómez Barry, H. — «Absceso hepático. Cuatro casos tratados con cloroquina», *Archivos del Hospital Universitario*, **3** (5): 453, Habana, Cuba, 1951.

# LES ASPECTS CLINIQUES DE L'AMIBIASE

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L'amibiase a une place à part en pathologie humaine. Elle n'y a pas d'équivalent. C'est abusivement qu'on l'a assimilée à d'autres maladies parasitaires générales ou locales. Tous les rapprochements entre amibiase et syphilis, amibiase et paludisme, amibiase et parasitoses intestinales sont biologiquement et cliniquement indus. L'amibiase ne peut pas être étudiée dans le cadre habituel des maladies infectieuses ou parasitaires et on ne peut, tentant de définir ses aspects cliniques et ses modalités évolutives, utiliser sans ambiguïté ou impropriété le vocabulaire de la pathologie générale. Sans une revue synthétique des grandes données de biologie parasitaire, de physiopathologie, d'anatomo-pathologie propres à l'amibiase il est impossible de la situer dans son cadre véritable, d'apprécier à la fois ses frontières anatomiques strictes, la simplicité de ses lésions et l'extension considérable des troubles fonctionnels qu'elle peut engendrer, impossible enfin d'édifier une nosologie et une nosographie pratiques, bases solides de toute étude clinique soucieuse d'objectivité.

C'est donc par un exposé succint de ce que nous appelons l'Amoebologie Générale qu'il convient de préluder à l'étude des aspects cliniques de l'amibiase.

Bien que morphologiquement et biologiquement très proche des multiples espèces telluriques libres et de différentes espèces parasites ou saprophytes des animaux, l'amibe dysentérique est étroitement adaptée à l'instestin terminal de l'homme, hors duquel elle ne peut vivre.

Le «complexe pathogène amibien» ne comprend que l'homme et le milieu extérieur.

Le mode de contamination est simple, il se résume en l'ingestion du kyste apporté par l'eau de boisson, les aliments souillés ou les mains sales, il est donc d'une simplicité redoutable.

Les formes de résistance, les kystes, rejetés avec les déjections fécales, ne poursuivent aucun développement dans le milieu extérieur. Ils y demeurent d'autant mieux et plus longtemps fertiles que l'humidité et la chaleur nécessaires à leur conservation sont mieux assurées, ce que réalisent parfaitement les climats des régions tropicales et équatoriales qui, toutes, représentent des territoires plus ou moins importants d'endémie amibienne. Les contaminations y sont à peu près inévitables, les recontaminations fréquentes.

Dans les régions tempérées, où les facteurs climatiques sont moins favorables à la survie des kystes, l'amibiase est demeurée sporadique sans tendance à se généraliser malgré des apports incessants.

Nous ne sommes pas encore absolument fixés sur le mode de libération de l'amibe à partir du kyste ingéré.

Les amibes tétragènes vivent à la surface de la muqueuse colique dans le mucus qui la recouvre et dans la lumière intestinale au milieu du bol fécal. Elles phagocytent des débris alimentaires et des bactéries et se divisent activement par scissiparité, puis s'enkystent après un certain nombre de divisions. Du kyste ingéré à la présence de kystes dans les selles 6 à 8 jours sont écoulés (Walker et Sellards).

L'individu qui a absorbé des kystes d'*Entamoeba dysenteriae* est devenu un porteur de kystes. Son tube digestif héberge un germe saprophyte n'exerçant aucune prédation, aucune action spoliatrice, irritative, toxique, en un mot, dépourvu de tout pouvoir pathogène. Si le saprophyte ne porte à l'économie de l'hôte qui l'héberge aucun dommage, aucune réaction de l'hôte ne paraît s'exercer à son endroit. Le saprophytisme ne sera révélé que par la découverte de kystes à 4 noyaux dans les selles et encore faut-il savoir que cette présence n'est pas permanente, l'élimination kystique obéissant à une rythmicité que traduisent des périodes muettes dont la durée atteint parfois 14 jours (Mathis).

L'amibe tétragène, comme tous les rhizopodes, est douée par sa division binaire d'un pouvoir de reproduction quasi-illimité. Peut-être la senescence, si l'apport extérieur ne se renouvelle pas, est-elle capable d'enrayer à la longue le développement de la lignée amibienne. Le saprophytisme amibien du bol fécal peut donc persister indéfini-

ment pour peu que les conditions physico-chimiques et biologiques du milieu intestinal lui soient favorables ou que, ce qui est banal dans les régions d'endémie, les réinfestations kystiques venues de l'extérieur le renouvellent.

Dans les régions tempérées où la permanence des réinfestations n'est pas assurée, la stérilisation du saprophytisme amibien peut se voir au bout de quelques mois ou de quelques années, mais même dans ces régions les persistances indéfinies du saprophytisme ne sont pas rares. Mathis avait invoqué, pour expliquer cette éventualité, la possibilité d'une auto-infestation comme dans l'oxyurose.

Cette conception d'un strict saprophytisme colique par l'amibe tétragène, simple culture dans le milieu fécal et le mucus ne s'accompagnant d'aucune lésion pariétale, d'aucun signe clinique, réalisant le «porteur sain de kystes» (Mathis), «l'amibiase-infestation» (F. Blanc), n'est pas acceptée par tous les auteurs. Il est cependant un fait biologique et clinique vérifiable aisément tous les jours, difficilement réfutable: de nombreux individus qui éliminent des kystes à 4 noyaux n'ont jamais présenté le moindre signe ou symptome d'amibiase colique et n'en présenteront jamais, d'autres non moins nombreux éliminent des kystes pendant des années avant de devenir des malades.

À l'opposé de cette opinion, Wenyon et O'Connor pensent qu'il n'y a pas de saprophytisme véritable, des lésions coliques très discrètes étant toujours trouvées lors de vérifications nécropsiques fortuites chez les porteurs de kystes (Faust).

C'est là un sujet de discussions qui n'est pas que doctrinal, car il domine la capitale question de la modalité du traitement préventif des rechutes et de la prophylaxie médicamenteuse de l'amibiase.

C'est à partir de cette infestation du bol fécal et du mucus par l'amibe tétragène, véritable réservoir de germes non encore pathogènes mais en puissance de le devenir, que se produisent les inoculations de la paroi colique quand, sous des influences locales ou générales diverses, ses résistances défaillent. La grande loi de pathologie générale qui veut que les organismes se défendant mal soient volontiers envahis par les agents d'agression morbides se vérifie, le saprophytisme devient un parasitisme, l'infestation une maladie.

Toutes les adultérations, fragilisations, pertes d'intégrité physique et fonctionnelle de l'épithélium colique normalement infranchissable à l'amibe sont autant de causes favorisantes de l'amibiase-maladie:

colites microbiennes banales ou spécifiques, intoxications médicamenteuses à déterminisme colique (purgations, mercure, sel d'émétine, etc.), perturbations vaso-motrices et neuro-végétatives, plus rarement traumatismes péritonéaux lors d'interventions chirurgicales, etc.

*À la première place de ces causes locales ou générales se situe l'infection microbienne, banale ou spécifique du colon.* Sévère et durable, elle permet les inoculations amibiennes pariétales massives qui sont à la base des amibiases coliques graves par l'importance de leur symptomatologie fonctionnelle et de leurs signes physiques et par leur tendance à lévolution prolongée ou à la rechute à court terme. *C'est cette fréquence des colites microbiennes qui explique l'incidence et la gravité des amibiases coliques dans les régions tropicales. Il sied ici de remarquer que l'autochtone plus ou moins immunisé à l'endroit des colites microbiennes par les atteintes multiples subies tout au long de son existence est, infiniment moins que l'Européen importé, une victime de la maladie amibienne et que, s'il demeure un redoutable semeur de kystes, il est bien moins souvent un malade. L'importance épidémiologique de ce fait ne saurait échapper.*

Les mouvements vaso-moteurs dûs au froid, les intoxications alimentaires au sens le plus large du terme sont les autres causes locales les plus fréquemment rencontrées. Sans gravité, de brève durée, elles se soldent par des pénétrations pariétales amibiennes peu nombreuses, non réitérées. L'épisode morbide sera sans gravité et de courte durée, sans tendance à la rechute rapprochée. C'est là, avec des exceptions toutefois, le fait de l'amibiase autochtone.

Les causes de débilitations générales: paludisme viscéral évolutif, tuberculose, syphilis, troubles nutritionnels (sauf l'avitaminose PP), ne paraissent pas intervenir dans le déterminisme des amibiases coliques graves, si tant est qu'elles interviennent, leur action n'est jamais au premier plan.

L'éthylisme est, par contre, un facteur très important de gravité, très souvent retrouvé dans les amibiases de longue durée.

La morphologie et les caractères biologiques de l'amibe ayant pénétré la paroi colique sont modifiés. La variété parasitaire du protiste s'écarte singulièrement de la variété saprophytique, *Entamoeba dysenteriae* variété *histolytica* est plus grande, plus fragile qu'*Entamoeba dysenteriae* variété *tetragena,* elle est en division active, phagocyte les globules rouges et a perdu son pouvoir d'enkystement.

Tous ces profonds changements qui traduisent l'adaptation à la vie parasitaire ne sont ni progressifs, ni lentement acquis, ce qui se concevrait facilement; ils sont au contraire soudains, quasi instantanés, comme une véritable mutation. Aucune des explications données de ce phénomène singulier n'est ni claire, ni satisfaisante.

*Entamoeba histolytica* franchit la barrière épithéliale en passant entre les cellules de revêtement, pénètre activement dans la sous-muqueuse, frappant de mort tous les éléments tissulaires à son contact, déterminant un abcès, un afflux leucocytaire local ajoute à la tension de l'abcès qui s'ouvre enfin dans la lumière intestinale.

À l'abcès succède l'ulcération où l'amibe poursuit son action nécrosante. La lésion fondamentale de l'amibiase est constituée.

C'est là la seule expression de l'agressivité de l'amibe histolytique. *L'amibe ne peut faire que de la nécrose, là se borne son pouvoir pathogène.* Mais, abcès nécrotique et ulcération siègent dans la paroi d'un organe creux anatomiquement et fonctionnellement complexe, secrétoire, mobile, absorbant, richement innervé et vascularisé, au contenu extraordinairement septique, et vont commander à tout un ensemble de désordres: douleurs diverses (tranchées, coliques, épreintes), hémorragies, muccorhée, perturbations neuro-végétatives locales ou à distance qui, se combinant diversement, donnent à l'amibiase colique ses différentes physionomies cliniques.

Les ulcérations récentes engendrent surtout des syndromes cliniques où dominent les phénomènes douloureux, les hémorragies, la muccorhée. Nombreuses et étendues, elles seront responsables des syndromes dysentériques typiques. Plus rares, plus limitées, elles commandent à des états morbides moins nets, plus simplement diarrhéiques.

L'amibe histolytique est dans la paroi colique et y demeure. Par voie portale, où elle accède par nécrose vasculaire dans le fond de l'ulcération, elle peut atteindre le foie et y exercer son activité nécrosante.

*L'amibiase est cantonnée au couple colo-hépatique. Seule une défaillance du filtre hépatique peut permettre le passage de l'amibe. Le poumon, filtre secondaire, joue alors son rôle. C'est au niveau de son parenchyme que les phénomènes de nécrose cellulaire, dûes au contact direct de l'amibe, se produiront alors créant l'abcès du pou-*

*mon. Si, par un hasard extraordinaire, l'amibe, après avoir franchi la barrière hépatique, passe encore la barrière pulmonaire, elle ne pourra donner que de grossiers abcès au niveau du parenchyme où elle embolisera (cerveau, reins, etc.) et de telles atteintes seront nécessairement très rares.*

La durée de la colite amibienne est extrèmement variable. Elle dépend de la durée et de la gravité de la méiopragie colique, de l'état antérieur du colon, de la valeur de la thérapeutique instituée. À la mort de l'amibe histolytique, le processus nécrotique s'arrête, l'ulcération demeure, torpide, une riche flore microbienne pullulant dans ses parois anfractueuses et retardant une guérison qui n'est obtenue, plus ou moins lentement, que par cicatrisation conjonctive, la complexité de la paroi colique s'opposant à une régénération tissulaire intégrale.

Les ulcérations déshabitées de leurs amibes et les lésions cicatricielles indélébiles qui les suivent peuvent commander à un ensemble plus ou moins important de symptomes fonctionnels et de signes physiques:

*a*) Perturbations du transit intestinal de cause directement anatomique, mais bien plus fréquemment fonctionnelle d'origine neuro-végétative;
*b*) Troubles avitaminosiques par difficulté de l'absorption, fuite vitaminique ou compétition microbienne;
*c*) Phénomènes de répercussivité à distance (gastriques, vasculaires, etc.).

La combinaison variable de ces troubles réalise des tableaux cliniques composites et souvent difficiles à reconnaître et à différencier de ceux entrainés par les ulcérations actives. Il va sans dire que ces tableaux seront d'autant plus importants que les ulcérations et leurs cicatrices seront plus nombreuses. Des ulcérations rares pouvant être cliniquement muettes.

Par ailleurs, tant que durera la tenace infestation de nouvelles agressions du colon seront toujours possibles et ce, d'autant plus facilement qu'un colon parsemé de cicatrices plus ou moins nombreuses, plus ou moins importantes, a perdu son intégrité fonctionnelle et se défend nécessairement moins bien qu'un colon normal et que

les ulcérations non entièrement guéries entretiennent l'infection microbienne, cause essentielle de la méiopragie colique.

Au terme de ces quelques réflexions l'amibiase nous apparait ce qu'elle est réellement: une maladie phylogénétiquement très simple, voire grossière, situé au plus bas de l'échelle nosologique, une maladie primitive au sens que Nicolle donnait à cette expression. Étroitement confinée par l'anatomie au couple colo-hépatique qu'elle peut rarement déborder vers l'appareil pleuro-pulmonaire et plus exceptionnellement vers d'autres organes, l'amibiase est essentiellement l'effraction d'une paroi colique le plus souvent déjà malade et qui se défend mal par un parasite à peine différencié au pouvoir pathogène limité à la seule nécrose de contact.

Malgré ces caractères restrictifs, l'amibiase s'avère cliniquement et plus encore évolutivement compliquée.

*Elle n'est pas, comme on l'a dit si souvent, une maladie d'emblée chronique aux rechutes fréquentes. Elle est d'abord une infestation cliniquement silencieuse, tenace, de longue durée, souvent définitive. Elle est ensuite, éventuellement, une succession d'accidents pathologiques locaux strictement coliques, plus ou moins nombreux, de survenue imprévisible, d'expressions cliniques diverses, de durée variable, tous susceptibles de léser définitivement l'intestin terminal et de se compliquer de métastases hépatiques.*

L'évolution de l'amibiase ne peut obéir à aucune loi, à aucune règle fixe. Il n'y a pas dans l'amibiase d'infestation, d'incubation, d'état aigu, d'état chronique, de rechutes selon les acceptions habituellement données à ces expressions.

On ne peut parler d'infestation amibienne comme on parle d'infestation paludéenne ou d'infection tuberculeuse ou brucellienne. Ce n'est pas dans un gîte viscéral profond, ganglionnaire, splénique ou médullaire comme c'est l'occurrence pour un «corps bleu» de Short, un bacille de Koch, une brucella, que demeure l'amibe pendant les silences cliniques, mais bien dans le bol fécal et le mucus à l'abri de toutes réactions de défense de l'organisme, c'est-à-dire pratiquement dans le milieu extérieur, mais un milieu extérieur protégé.

Qu'est-ce que l'incubation de l'amibiase? Quelle est sa durée?

Tel sujet pendant des années éliminera des kystes à 4 noyaux avant de devenir un malade, tel autre présentera des troubles coliti-

ques graves dès les premiers instants de sa contamination (éventualité extrèmement banale en Asie Tropicale, où le même aliment souillé apporte des kystes à 4 noyaux et le microbe de la colite).

Les rechutes sont commandées par les mêmes mécanismes pathogéniques que les atteintes initiales. Les réactions de l'organisme antérieurement frappé ne diffèrent en rien de celles de l'organisme neuf. Rien qui puisse faire envisager immunité ou prémunition ne peut être cliniquement constaté. L'accident initial colique ne diffère en rien anatomiquement et pathogéniquement des rechutes plus ou moins nombreuses, plus ou moins espacées, qui pourront le suivre. Le terrain sera toujours le même, le parasite, renouvelé à la source de l'infestation, ne manifestera jamais le vieillissement générateur de modalités cliniques torpides.

Même la division simple en formes aiguës et chroniques est inadéquate quand on veut l'appliquer à l'amibiase.

Quest-ce qu'une amibiase colique aiguë?

Est-ce une amibiase à la symptomatologie fonctionnelle et aux signes physiques graves, qui, évoluant en quelques jours ou quelques semaines, doit être opposée à l'amibiase colique chronique aux signes et symptomes moins bruyants et dont l'évolution s'étale sur des mois, voire des années?

Il est certes des amibiases coliques dysentériques ou diarrhéiques à la symptomatologie fonctionnelle marquée et aux signes physiques importants qui évoluent en quelques jours. Elles représentent d'authentiques amibiases aiguës.

Il est d'autre part des états coliques amibiens aux signes physiques à peu près nuls, aux symptomes fonctionnels discrets aisément tolérés pendant parfois plus de vingt ans. Les dyschésies entrent parfaitement dans ce cadre. Ce sont là réellement des amibiases qui méritent l'appellation de chronique.

Mais il est des amibiases et combien nombreuses en régions tropicales (nous n'osons écrire les plus nombreuses) qu'il est impossible de classer raisonnablement dans ce dyptique acuité-chronicité. Où situer la dysenterie aiguë, voire même suraiguë (plus de 20 selles, douleurs abdominales très vives), si banale en Extrême Orient, qui, littéralement cachectisante, va évoluer pendant des semaines, voire des mois, sinon plus d'une année, résistant aux thérapeutiques ou à peine influencée par elles? Comment dénommer cette forme gra-

vissime? Amibiase colique chronique ou amibiase colique aiguë? Si l'acuité des symptomes appelle l'adjectif aigu, la longue évolution commande l'adjectif chronique. Et les mêmes réflexions pourraient être faites à propos de certains états diarrhéiques graves.

Classer sous la même rubrique chronique la dysenterie qui évolue inexorablement pendant 12 mois, la diarrhée au long cours avec lente dénutrition et la dyschésie allègrement tolérée pendant 20 ans est paradoxal.

Il n'est pas davantage satisfaisant pour l'esprit de mettre côte à côte dans les formes aiguës une diarrhée qui se porte sur pied et guérit en trois jours et la dysenterie qui pendant plus d'un an tourmente un grabataire.

Jusqu'au test étiologique qui est en défaut. Il n'est pas possible de concevoir une classification de quelque valeur pratique basée sur les différentes formes parasitaires, les états aigus étant caractérisés par la présence d'amibes hématophages dans les selles, les états chroniques par la présence des formes tétragènes ou kystiques, les états séquellaires par l'absence de toute forme parasitaire.

La présence d'amibes hématophages dans les selles signifie qu'il existe quelque part dans la paroi colique un processus ulcératif en activité, ce qui est aussi bien le fait de l'amibiase la plus suraiguë que de la plus torpide.

Cette présence de l'amibe hématophage n'est d'ailleurs pas toujours confirmée aisément par le laboratoire et, sans être contingente, elle n'est pas indispensable à l'affirmation d'une étiologie amibienne au cours de certains états qui cliniquement s'imposent comme amibiens.

Quant aux kystes à 4 noyaux et aux formes végétatives, leur présence traduit une infestation amibienne, elle n'affirme pas péremptoirement la nature amibienne d'un état intestinal chronique qui peut être dû à toute autre cause. Nous savons d'ailleurs l'existence des phases muettes dans l'émission des kystes et la relative créance à accorder au résultat négatif d'examens non répétés en série.

Dans les traités de médecine tropicale ou de médecine interne, au chapitre de l'amibiase, les grandes divisions anatomiques en amibiase colique, hépatique, pulmonaire se retrouvent toujours, nécessairement. Les aspects cliniques relativement simples des localisations hépatiques et pulmonaires sont désignés avec clarté — encore que la

précision ne soit pas toujours idéale en ce qui concerne l'amibiase hépatique dont le stade lésionnel n'est nullement déterminé par les expressions de congestion du foie, d'hépatite pré-suppurative —, mais la plus grande confusion règne trop souvent à propos des aspects cliniques et des modalités évolutives de l'amibiase colique. Les acceptions accordées aux mots *aigu, chronique, séquelles* sont variables sans netteté. Chaque auteur a son vocabulaire. Le paragraphe dit des «complications» clot le chapitre et renferme à coté des manifestations morbides dûment amibiennes: cutanées, spléniques, cérébrales, etc., des états pathologiques tels que néphrites, bronchites, etc., qui pour beaucoup d'entre eux n'ont jamais rien eu à voir avec l'amibe dysentérique.

Cette absence de rigueur rend difficiles et souvent vaines les discussions à propos de pathologie et de thérapeutique amoebologiques. Beaucoup d'auteurs ne se comprennent pas qui ne parlent pas la même langue.

Il serait éminemment souhaitable qu'une codification de la nosologie et de la nosographie amibiennes soit envisagée. Ce qui a été fait pour la lèpre doit pouvoir l'être pour l'amibiase.

À chaque manifestation morbide dûe au bacille de Hansen une appellation plus ou moins longue mais toujours simple peut être accolée. Non seulement elle désigne sans ambiguïté l'aspect clinique et la modalité évolutive, mais elle implique, dans une large mesure, le pronostic et les indications thérapeutiques.

Il est, pensons-nous, possible d'obtenir des précisions d'un ordre comparable en matière d'amibiase.

Quatre problèmes sont en fait à considérer.

Seuls les deux premiers, difficiles, méritent un long développement.

Nous envisagerons ainsi successivement:

Les aspects cliniques et la nosographie de l'amibiase colique;

Les aspects cliniques et la nosographie de l'amibiase hépatique;

Les aspects cliniques et la nosographie de l'amibiase pleuro-pulmonaire et plus largement respiratoire;

Les aspects cliniques et la nosographie de l'amibiase extra-colo-hépato-respiratoire.

## ASPECTS CLINIQUES ET NOSOGRAPHIE DE L'AMIBIASE COLIQUE

Un système de références à partir des caractéristiques séméiologiques, évolutives, anatomiques les plus simples et les moins discutables peut permettre d'individualiser avec le maximum de précision accessible à la clinique et à la biologie quelque moment que se soit de l'amibiase colique et de le situer à sa place exacte dans une classification cohérente.

Une amibiase colique peut parfaitement être définie par:

*a*) Son mode d'apparition:

Première atteinte;
Rechute éloignée de l'atteinte précédente;
Rechute rapprochée de l'atteinte précédente.

*b*) Sa symptomatologie fonctionnelle prédominante:

Diarrhée;
Dysenterie;
Dyschésie.

*c*) Sa durée brève ou longue.

Des caractères secondaires sont susceptibles le cas échéant d'apporter une précision complémentaire:

Origine géographique;
Limitation au colon ou extension au foie;
Prédominance des ulcérations, coecale, rectale, transverse bi-polaire;
Particularité clinique marquante:

Douleurs excessives;
Avitaminose secondaire;
Association microbienne spécifique, etc.

De ces coordonnées la plus importante est la durée.

Les amibiases coliques évoluant d'une seule poussée prolongée ou procédant par rechutes à court terme, voire subintrantes, sont souvent des amibiases graves par l'importance de leur symptomatologie fonctionnelle diarrhéique ou dysentérique.

À cette gravité clinique s'ajoute essentiellement pour les formes dysentériques l'entrave que leur modalité évolutive apporte à la mise en jeu d'une thérapeutique efficace. L'amibiase de longue durée ne peut recevoir les traitements spécifiques suffisamment prolongés qu'elle requiert mais qu'interdit la toxicité des amoebicides. La rechute à très court terme (moins de 30 jours) ne peut bénéficier d'un traitement spécifique répété qui se heurte à la toxicité accumulative du traitement précédent (nous rappellerons que l'élimination de l'émétine demande 45 jours).

Les amibiases coliques de brève durée sont, au contraire, le plus souvent des amibiases bénignes par la discrétion de leur symptomatologie fonctionnelle. Leur modalité évolutive n'apporte aucun obstacle à l'administration des traitements spécifiques.

Le défaut d'une telle nosologie et d'une telle classification réside dans la multiplicité des formes dénombrées et dans les appellations peut-être trop détaillées. Mais est-il possible de faire autrement et peut-on reprocher la recherche d'un maximum de précision?

Dire d'une amibiase colique qu'elle est de première atteinte, diarrhéique, sans participation hépatique, de brève durée, d'origine autochtone ou qu'elle est de rechute rapprochée, dysentérique, avec participation hépatique, de longue durée, d'origine indochinoise est aussi satisfaisant pour l'esprit et singulièrement plus précis et plus évocateur que de parler d'amibiase aiguë ou de dysenterie amibienne chronique sans plus.

Les états coliques séquelles chez les amibiens et les manifestations à distance de l'amibiase colique ou de ces séquelles complètent cette nosographie.

*Les états coliques séquelles chez les amibiens* sont l'ensemble des symptomes fonctionnels, des signes physiques, des symptomes généraux dont le substratum organique réside dans les lésions coliques déshabitées à tout jamais de leurs amibes (amibiase colique guérie et infestation stérilisée) et dans les cicatrices qui suivent ces lésions.

C'est un chapitre complexe aux limites floues que nous essaierons de préciser.

*Les manifestations à distance de l'amibiase colique ou de ses séquelles* sont les retentissements neuro-végétatifs qui, à partir d'excitations nées dans les ulcérations actives ou leurs séquelles, s'étendent bien au delà, par répercussivité, du cadre colique et même de la cavité abdominale.

Nous ne pouvons décrire tous les aspects cliniques de l'amibiase colique. Ce serait fastidieux, sans intérêt véritable, singulièrement hors des proportions de ce travail. Le nombre de ces aspects est théoriquement et pratiquement considérable, mathématiquement déterminé dans notre classification.

Nous rappellerons brièvement quelques-uns d'entre eux parmi les plus importants.

### Amibiase colique de brève durée à forme diarrhéique ou dysentérique.

Ce sont le plus souvent des amibiases d'origine métropolitaine ou contractées en Afrique Mineure ou dans le bassin Méditerranéen. Il s'agit de sujets infestés depuis plus ou moins longtemps qui, amoindris dans leur résistance colique, deviennent des malades. Les causes, labiles, de la transitoire défaillance des résistances coliques sont rarement retrouvées à l'examen ou à l'interrogatoire.

Le tableau clinique est le plus simple: Syndrome dysentérique moyen ou plus banalement syndrome diarrhéique, cinq à dix selles par jour. Peu de signes fonctionnels hors la doleur colique permanente, discrète le plus souvent et qu'exagèrent les efforts d'expulsion. Les signes physiques sont réduits à la spasticité d'un cadre colique, érectile sous la main qui palpe et douloureux au niveau du coecum et du sigmoïde. Peu de signes généraux, pas de fièvre. Les constantes biologiques ne sont pas perturbées. La formule leucocytaire est normale. La V.S.H. est normale, son augmentation signifierait une atteinte hépatique, métastase que l'examen clinique ne pourrait manquer de révéler: hypertrophie modérée du viscère, douleurs à l'ébranlement. réaction basale droite.

L'amibe histolytique est aisément trouvée dans la glaire dysentérique ou dans les parcelles muco-sanglantes de la selle diarrhéique.

Du tableau clinique aucune autre indication thérapeutique ne se dégage que la nécessité de détruire l'amibe histolytique, ni lutte contre une infection microbienne associée, ni correction d'un état neuro-végétatif ou nutritionnel.

L'émétine à posologie classique est le médicament de choix.

Le traitement par les arsenicaux ou les oxy-quinoléines iodiques que la simplicité de son administration orale fait volontiers mettre en œuvre peut entrainer une guérison clinique, voire une guérison anatomique, les lésions superficielles étant parfaitement atteintes et les amibes à leur niveau par ces médicaments non diffusibles agissant par contact (amoebicides de contact), mais le péril d'une métastase hépatique méconnue que ce traitement ne saurait atteindre est une réalité avec laquelle il faut toujours compter. Des réflexions comparables peuvent être faites en ce qui concerne les abiotiques qui, améliorant les conditions microbiennes coliques, permettent en fait une guérison spontanée de l'amibiase.

Les résultats du traitement correct d'une forme bénigne diarrhéique ou dysentérique sont toujours bons.

Les rechutes sont imprévisibles. Elles pourront fort bien ne pas se produire si le traitement a été précocement entrepris et il n'y a pas lieu d'envisager leur prévention par des cures arsenicales ou oxy-quinoléiques suivant le traitement émétinien.

### Amibiase colique de longue durée à forme dysentérique.

Elles sont le plus souvent, sinon toujours, d'origine tropicale. La pathogénie des amibiases dysentériques graves est dominée par le rôle de l'infection microbienne sévère et durable, qu'elle soit banale ou spécifique. La paroi colique, fragilisée par l'atteinte microbienne, n'offre aucune résistance à l'agression amibienne, qui, étendue et massive, sans cesse renouvelée à partir du réservoir de virus du bol fécal et du mucus, adultère gravement le colon, assurant ainsi la perennisation de la colite microbienne. Le cercle vicieux est fermé dont sortira difficilement le malade.

La maladie s'est installée soit brusquement, première manifestation ou rechute isolée, soit, au contraire, progressivement, à la suite

de rechutes de plus en plus rapprochées jusqu'à en devenir subintrantes et chez des malades dont le traitement a été ambulatoire, fait trop banal chez le soldat en campagne de guerre tropicale.

Le tableau clinique est souvent impressionnant, 20 à 30 selles par jour sanglantes et muqueuses ou séro-sanglantes, douleurs intenses de tout le cadre colique exagérées lors des efforts d'expulsion et exacerbées par la palpation même prudente.

Dans les selles les amibes histolytiques fourmillent. Une note fébrile modérée est fréquente, même avec foie indemne, la participation hépatique est d'ailleurs contingente et se traduit comme toujours par l'hypertrophie de la glande, douloureuse spontanément et à l'ébranlement, l'augmentation de la V.S.H. de la leucocytose.

Le traitement spécifique se heurte à l'obstacle de la toxicité accumulative émétinienne ou conessinique qui interdit les cures prolongées que nécessite la longue évolution ou la reprise des cures rapprochés que commandent les rechutes précoces.

Avant l'ère des abiotiques aucun moyen de lutte sérieux ne pouvait être opposé à la colite microbienne que seul le rapatriement était susceptible d'améliorer (suppression des apports microbiens).

Le double traitement abiotique et amoebicide amène des guérisons fréquentes et si la rechute se produit elle est très souvent rejetée au delà de la période de toxicité accumulative et son traitement ne présente pas de difficultés particulières.

### Amibiase colique de longue durée à forme diarrhéique.

Un état diarrhéique qui se prolonge plus ou moins bien toléré est une des expressions cliniques et évolutives les plus banales des atteintes amibiennes coliques tropicales. Elle est très fréquente chez les rapatriés. Dans les contaminations métropolitaines, elle est rare, sans être cependant exceptionnelle, et elle y est souvent méconnue. Son début est toujours insidieux: diarrhée à forme dysentérique de gravité moyenne insuffisamment traitée, syndrome diarrhéique d'emblée dont la nature amibienne n'est soupçonnée qu'après une longue évolution.

Plus rarement, la diarrhée succède à un état dysentérique grave de longue durée ou à rechutes rapprochées que le traitement a modifié.

La pathogénie des diarrhées d'origine amibienne de longue durée est complexe, faisant intervenir, isolément ou rassemblés, des phénomènes inflammatoires coliques, des troubles de la sécrétion muqueuse, des perturbations mécaniques et surtout neuro-végétatives, des avitaminoses.

Une diarrhée profuse et qui dure est un facteur important d'avitaminose PP et une des expressions mineures de la pellagre est la diarrhée. Il y a là un cercle vicieux qui nous parait être extrèmement fréquent dans les amibiases à forme diarrhéique.

L'association à l'amibiase d'une parasitose à flagellés (lamblias, tetramitus, etc.) a pu être invoquée c'est une interprétation fausse. La pullulation du flagellé est une conséquence, non une cause, de l'état diarrhéique.

Une grande difficulté d'interprétation apparait dans tous ces états. S'agit-il, si l'amibe n'est par découverte, d'une amibiase à forme diarrhéique ou d'un état séquelle chez un amibien?

La réponse est malaisée à donner. Il faut cependant remarquer que très fréquemment les amibes sont découvertes et que le traitement spécifique par l'émétine, même dans les états d'évolution très ancienne, n'est pas dépourvu d'action au moins passagère. Il est fréquent de noter que la plupart de ces malades, sinon tous, sont des hypervagotoniques.

La vitaminothérapie PP à doses importantes, 0,60 g à 1 g par jour, par voie parentérale, jointe au traitement par abiotiques et amoebicides, donne des résultats souvent remarquables après l'échec de toutes les autres thérapeutiques.

### Amibiase colique de longue durée à forme diarrhéique avec troubles nutritionnels.

Il s'agit presque toujours d'états diarrhéiques succédant à une amibiase de type dysentérique de longue durée ou à rechutes rapprochées ou d'états diarrhéiques installés d'emblée évoluant d'une seule tenue, mal traités, et le plus souvent sans la prescription impérative du repos. Des traitements par antibiotiques prolongés sont souvent retrouvés dans les antécédents. Amaigrissement, anémie, pigmentation cutanée, troubles muqueux composent un tableau singulièrement voisin de celui de la sprue, seuls le régime et l'aspect des selles

(4 à 5 selles liquides fécaloïdes et muqueuses), l'anémie non macrocytaire, la réaction normoblastique de la moelle permettent de tracer une frontière quelque peu arbitraire entre ces états diarrhéiques et la sprue et ce, d'autant plus, que des sprues les plus légitimes cliniquement et hématologiquement apparaissent comme d'incontestables complications des amibiases de longue durée à forme diarrhéique.

Le traitement qui est celui des amibiases de longue durée à forme diarrhéique fait intervenir la vitamine PP à forte dose. Les résultats en sont généralement bons.

La vitamine $B_{12}$ a son indication dans les cas s'accompagnant de mégaloblastose. Mais il s'agit alors de sprue authentique.

### Amibiase de longue durée à forme dyschésique.

La dyschésie apparaît le plus souvent à la suite d'une amibiase dysentérique de première atteinte ou de rechute aux symptomes fonctionnels et aux signes physiques peu marqués et dont le traitement spécifique a eu rapidement raison. Le retour d'un transit intestinal normal ne sanctionne pas la guérison. Le malade entre dans un état de constipation tenace. Parfois c'est longtemps après la guérison d'un épisode dysentérique suivi de rechutes éloignées à forme diarrhéique et de brève durée que le transit intestinal se modifie et que la constipation s'installe.

Le malade émettra désormais une seule selle tous les 3 ou 4 jours, voire toutes les semaines: moule rectal volumineux d'une seule pièce, vernissé et luisant avec quelques glaires sanguinolentes terminales: le cachet dysentérique des vieux auteurs. Hors cette expulsion, véritable accouchement dont les douleurs sont souvent vives, le malade ne se plaint de rien. Il est floride, actif, l'appétit conservé. Tous les grands appareils sont indemnes.

À l'examen de l'abdomen, on note des cordes coliques spastiques.

La rectoscopie révèle un aspect normal.

Les examens de laboratoire sont sans grand intérêt. Les amibes peuvent parfois, difficilement, être mises en évidence dans les glaires terminales, où de rares formes histolytiques sont rencontrées après de nombreux examens négatifs.

Ces dyschésies durent des années, immuables dans leurs signes et symptomes, sans entrainer jamais la moindre adultération de l'état

général. Des débâcles diarrhéiques, émission de 5 à 6 selles liquides et glaireuses, interrompent plus ou moins régulièrement la constipation opiniâtre.

La pathogénie de cet état dyschésique amibien est assez difficile à concevoir. Peut-être s'agit-il de spasmes coliques permanents entretenus par des ulcérations haut situées et hors de portée du rectoscope chez des sujets sympathicotoniques. Il est de fait que la plupart des dyschésiques sont des sympathicotoniques ou des amphotoniques. L'examen radiologique du colon ne montre pas toujours, tant s'en faut, l'existence de ces spasmes.

Le traitement des états dyschésiques mérite qu'on s'y arrête. Il est remarquablement réalisé par l'émétine à faible dose, 2 cg par jour en injection sous-cutanée, sans adjonction de strychnine et par le gardénal à doses filées, une prise de 0,01 à 8 h, 12 h, 16 h, 20 h et au moment du coucher.

Il est rare qu'il faille recourir à plus de 10 jours de ce traitement pour amener une guérison totale de cet état de constipation si particulier qui avait résisté aux thérapeutiques habituelles.

L'adjonction au gardénal de chlorydrate de papavérine 0,04, 4 à 5 fois par jour, est une bonne mesure dans les cas où les phases de constipation alternent nettement et régullièrement avec les phases de diarrhée.

C'est dans l'action tissulaire de l'émétine beaucoup plus que dans son action amoebicide qu'il faut voir l'explication de ces guérisons souvent remarquables.

Au niveau de l'ulcération, la fibre lisse décontractée, mise au repos, cesse d'être la source d'excitation permanente. L'arc réflexe est ainsi coupé à son origine, aussi bien qu'il l'est au niveau de ses voies afférentes et efférentes par les médicaments neurolytiques.

### Deux aspects cliniques importantes: les localisations coecales et rectales.

La localisation prédominante des ulcérations au niveau du rectum et du coecum permet d'individualiser légitimement une forme rectale et une forme coecale de l'amibiase. Elles sont aussi bien le fait des amibiases de longue durée que de brève durée.

*La forme rectale.* — Elle est caractérisée par le ténesme à irradiation vésicale susceptible d'entraîner des erreurs de diagnostic. Elle se voit volontiers chez l'enfant. Le toucher rectal révèle une muqueuse boursouflée et granuleuse. La rectoscopie est extrèmement pénible et il vaux mieux l'éviter. Les hémorroïdes et un prolapsus muqueux sont des complications fréquentes. Lors des ténesmes les plus vifs, les plus intenses, le sphincter est parfaitement continent et il n'y a jamais de perte involontaire des matières, jamais de béance de l'anus comme dans la dysenterie bacillaire. L'extension à la marge de l'anus et au périnée du processus ulcératif est une complication redoutable, heureusement très rare.

Mal traitée, parce que méconnue, la rectite amibienne peut se compliquer très exceptionnellement de retrécissement amibien. La striction est bas-située, à moins de 7 cm, et aisément accessible au doigt.

Aux traitements commandés par l'amibiase colique seront adjoints les pansements rectaux et même sigmoïdiens.

*La forme coecale.* — Elle est exceptionnelle dans les amibiases diarrhéiques. Elle est dans l'ensemble assez rarement rencontrée. Sa connaissance est d'une importance primordiale.

Deux types d'amibiases coecales peuvent être décrits:

L'amibiase coecale à forme pseudo-appendiculaire;
L'appendicite amibienne vraie.

*Amibiase coecale à forme pseudo-appendiculaire.*

Après un bref stade de diarrhée ou de dysenterie sans caractère de gravité particulier, apparaît brutalement une douleur atroce de la fosse iliaque droite, réellement anormale par son intensité. Le diagnostic d'appendicite aiguë généralement accepté sans discussion conduit à l'acte opératoire, dont nous verrons dans un instant les redoutables conséquences immédiates.

Un examen clinique soigneux et attentif permet toujours de reconnaître l'amibiase coecale dans sa forme pseudo-appendiculaire:

Le malade est généralement apyrétique;
Il n'existe pas de défense et de contraction musculaire véritable;
La douleur n'est pas exquise en un point précis,

mais, au contraire, étendue à tout le colon droit, particulièrement sur son bord externe. L'examen du malade dans le décubitus latéral gauche, jambes fléchies, permet d'apprécier aisément par une palpation douce cette prédominance douloureuse. La dépression de la paroi par la tranche d'une pièce de monnaie permet de dessiner le bord externe douloureux du colon. C'est là une vieille pratique séméiotique qui peut encore rendre des services.

Il n'y a pas de vomissements, simplement de l'émétisme. Très souvent le foie est légèrement hypertrophié et douloureux à l'ébranlement, la leucocytose est modérée, la formule leucocytaire est normale.

En milieu endémique amibien le malade sera mis en surveillance médicale et un traitement d'épreuve immédiatement institué. L'injection sous-cutanée de 12 cg d'émétine avec les correctifs nécessaires de strychnine, 4 mg, et de vitamine $B_1$, 100 mg, est sanctionnée, en quelques heures, par la sédation totale des phénomènes douloureux et de leur impressionant cortège.

Si aucun traitement spécifique n'est entrepris, en quelques jours, l'amibiase jette le masque et évolue sur un mode dysentérique grave.

L'intervention malencontreusement pratiquée entraine dans les jours qui la suivent une gravissime dysenterie très difficile à réduire. L'acte chirurgical a représenté une cause de fragilisation colique extrêmement importante.

*L'appendicite amibienne vraie.*

Le tableau est celui d'une appendicite aiguë banale, réaction pariétale, douleur élective, début brusque, température élevée. Peut-être le Douglas est-il moins douloureux au toucher que dans une appendicite ordinaire?

Un syndrome diarrhéique est assez banal dans l'appendicite aiguë pour ne pas faire envisager d'emblée une étiologie amibienne. Le syndrome dysentérique peut être davantage pris en considération.

Ce tableau clinique est le fait de l'appendicite amibienne vraie.

Les réactions de l'appendice sont les mêmes que la lésion soit un abcès nécrotique amibien ou un abcès folliculaire microbien. En l'une et l'autre occurrence étiologique les dangers de perforation et de péritonite sont les mêmes.

L'appendicectomie s'impose.

Les conséquences de l'étiologie amibienne apparaitront dans quelques jours et la dysenterie grave qui ne manque pas de s'installer sera justiciable des traitements habituels.

### Un aspect clinique particulier: par association morbide, la dysenterie amoebo-bacillaire.

Très fréquente en Extrême Orient et même ailleurs, parfaitement bien connue des anciens auteurs, elle est peut-être trop oubliée aujourd'hui. Les symptomatologies fonctionnelles et les signes physiques du syndrome dysentérique sont à leur maximum, l'état général est très profondément touché. C'est l'atteinte par le bacille dysentérique spécifique qui a permis la pénétration pariétale de l'amibe. Le porteur de kystes a contracté une dysenterie bacillaire, éventualité extrèmement fréquente dans les régions où les deux maladies sont endémiques (Grall).

Les lésions nécrotiques amibiennes s'ajoutent à la nécrose bactérienne. La résorption toxique est massive, génératrice de choc.

Il faut chez ces malades lutter contre l'intoxication, et ici nous croyons devoir faire une réflexion: l'intoxication est un fait clinique, non une constatation de laboratoire, et quel que soit le bacille dysentérique isolé en culture, la sérothérapie anti-dysentérique sera entreprise si les signes et symptomes d'intoxication sont menaçants.

Après 24 heures de traitement antibiotique et antitoxique, l'émétine sera administrée à la posologie habituelle et l'antibiothérapie continuée pendant une semaine.

Les résultats sont très bons.

### Les résultats séquelles chez les amibiens.

Les séquelles cicatricielles des ulcérations sont bien plus rarement, directement par action mécanique (endurcissement pariétal, rigidité, coudure, atrésie, brides, adhérences), responsables de syndrome sub-occlusif de sténose ou de diarrhée qu'indirectement par excitation permanente du système neuro-végétatif entrainant par voie réflexe les perturbations motrices secrétoires et douloureuses de la colo-névrite. C'est là un chapitre nosographique très mal précisable, car il est évi-

dent que les mécanismes et les conséquences de la colo-névrite sont les mêmes, que l'excitation émane d'une ulcération en activité, d'une ulcération déshabitée ou de la lésion cicatricielle qui la suit. Chez le même malade peuvent co-exister d'ailleurs et pendant fort longtemps les trois types de lésions.

Dans certains états diarrhéiques de longue durée, certaines dyschésies, il est bien difficile d'affirmer qu'il s'agit d'une amibiase en activité ou d'un état rigoureusement séquellaire. Les signes physiques et les symptomes fonctionnels sont les mêmes, la preuve étiologique est difficile à administrer par le laboratoire, les traitements d'épreuve sont contingents car l'émétine, le seul médicament valable dans ces occurrences, est à la fois un amoebicide et un remarquable calmant de la fibre lisse.

La discussion est au fond quelque peu byzantine.

### Les manifestations à distance.

Les phénomènes de répercussivité grèles, gastriques, cholécystiques et même respiratoires et cardio-vasculaires sont extrèmement fréquents dans les amibiases coliques de longue durée et dans les états coliques séquellaires. Ils donnent des tableaux variables et complexes selon les conditions neuro-végétatives, la prédominance vagale ou sympathique du malade.

Les troubles nutritionnels, les avitaminoses endogènes qui compliquent si souvent les colites amibiennes anciennes à forme diarrhéique sont peut-être à rapporter, en partie, à des difficultés de l'absorption par sidération fonctionnelle du grèle qui ajoute leurs effets à la fuite vitaminique.

Les syndromes gastro-duodénaux à symptomatologie grossièrement comparable à celle de la maladie ulcéreuse cèdent remarquablement au traitement émétinien, alors que les médications symptomatiques et à visées physiologiques dirigées contre la maladie ulcéreuse sont sans aucune action.

L'examen et surtout l'interrogatoire attentif des malades permettent assez vite de reconnaître la nature réflexe de ces troubles: absence d'horaires douloureux précis et de rythmicité prandiale, action paradoxale des régimes.

Il est également banal de rencontrer des réactions cholécystiques douloureuses qui ne sont ni le fait de calculs ni d'atteinte du cystique ni d'oddites, comme un tubage minuté et des examens radiologiques le peuvent montrer.

Les palpitants, les faux angoreux sont monnaie courante parmi les sujets entachés d'amibiase ancienne. L'intégrité des électrocardiogrammes, l'absence d'action des thérapeutiques sédatives, les heureux résultats immédiats d'un traitement émétinien rapportent rapidement à leur cause ces troubles très fréquents.

Plus largement enfin, une note asthénique et une extrême frilosité qui cèdent très vite, elles aussi, à l'émétine sont, dirions-nous, presque constantes.

Le diagnostic de tous ces troubles, qui représentent un chapitre à peine ébauché, est généralement facile; il utilisera l'histoire du malade où l'amibiase se retrouve toujours, la négativité des contextes organiques, la présence des cordes coliques et l'action souvent spectaculaires du traitement émétinien à faibles doses.

## ASPECTS CLINIQUES ET NOSOGRAPHIE DE L'AMIBIASE HÉPATIQUE

Les données pathogéniques de l'amibiase hépatique sont bien plus simples que celles de l'amibiase colique.

Au niveau du foie l'amibe dysentérique est seule en cause, sans action microbienne préalable ou associée. La destruction par nécrose d'un plus ou moins grand volume de parenchyme hépatique n'entraine aucun trouble fonctionnel, aucune perturbation neuro-végétative ou nutritionnelle.

Les problèmes nosologiques et nosographiques posés par l'amibiase hépatique ne sont pas pour autant simplifiés ni plus aisément résolus.

Il ne saurait exister d'amibiase hépatique sans lésion amibienne du colon:

Amibiase hépatique d'accompagnement quand l'atteinte colique est évidente;

Amibiase hépatique primitive quand l'atteinte colique est inapparente.

À partir de l'ulcération colique, les amibes drainées par le courant sanguin portal arrivent au foie, où leur arrêt dans les veines interlobulaires réalise un véritable thrombus parasitaire accompagné de réactions congestives. Cette phase intra-vasculaire peut représenter toute l'atteinte hépatique, si les résistances organiques sont efficaces ou si une thérapeutique spécifique est instituée. Mais, le plus souvent, il n'en est rien, le pouvoir cytolytique de l'amibe s'exerce alors sur la paroi de la veine, puis sur le parenchyme immédiatement voisin. L'hépatonécrose s'ajoute au thrombus (Brown, Councilmann, Lafleur, Dopter, Jorgens), la lésion initiale de l'amibiase hépatique est constituée. C'est l'îlot nécrotique élémentaire, le «point nécrotique primaire» (Palmer): granulation de 1 mm à 2 mm, magma de cellules hépatiques dégénérées, de leucocytes et d'amibes, bordée d'une mince bande de coagulum capillaire.

Le devenir de cette lésion dépend de la vitalité de l'amibe et des résistances spontanées ou thérapeutiques qu'elle va rencontrer. Si l'amibe meurt, l'organisme réalise sans effort l'effacement total du micro-abcès par cicatrisation conjonctive et surtout régénération cellulaire, la guérison est totale. Si les résistances spontanées sont insuffisantes, si aucune thérapeutique spécifique n'intervient, l'amibe prolifère dans le parenchyme hépatique et y exerce son action nécrotique, le minuscule foyer s'agrandit. Pour peu que plusieurs de ces centres de nécrose primaire entrent en coalescence, la cavité qu'ils dessinent s'étend, se creuse, pleine de ce que l'on appelle improprement le pus amibien. Une collection se développe plus ou moins vaste qui refoule les cellules hépatiques demeurées saines et le tissu de soutien. C'est l'abcès amibien du foie. Son évolution sera fixée par l'extension ou l'arrêt du développement des îlots nécrotiques élémentaires, par leur nombre, par la répétition des embols amibiens. Toutes les possibilités se peuvent concevoir, de la cavité limitée aux fusées migratrices les plus déconcertantes.

À lar mort de l'amibe, le processus nécrotique s'arrête mais la collection demeure, lentement remaniée par les jeux tissulaires de cicatrisation et de régénération cellulaire. La disparition du magma est d'autant plus lente que le volume de l'abcès était plus grand. Le «pus amibien» passe par des aspects différents longuement décrits par les classiques: chocolat, café au lait, mastic — aspects de moins en

moins fréquemment rencontrés à notre époque de traitement spécifique — avant d'atteindre à l'état fibreux et crétacé des cicatrisations définitives.

Chez les mêmes malades peuvent coexister un semis plus ou moins important d'îlots nécrotiques primaires, isolés ou disséminés, et de cavités nécrotiques plus ou moins volumineuses. Aucune barrière n'existe entre les deux processus. Il n'y a pas plus de différence entre la nécrose hépatique en îlots primaires isolés et disséminés et la nécrose collectée en abcès de volume variable qu'il n'y en a entre l'infiltrat tuberculeux et la caverne. Comme la matière tuberculeuse est une, la matière amibienne est une, qu'on nous permette cette comparaison un peu osée. Imagine-t-on un phtisiologue essayant, sans l'auscultation et l'examen radiologique, de faire le bilan lésionnel d'un tuberculeux pulmonaire? C'est pourtant la situation rigoureuse du médecin devant le foie amibien. Il existe bien des abcès amibiens du foie dont le diagnostic s'impose à l'évidence clinique ou radiologique, mais les atteintes hépatiques amibiennes dont il est impossible de reconnaître d'emblée le stade lésionnel sont bien plus nombreuses.

L'abcès profond, unique ou multiple, et le semis de micro-abcès de la nécrose en îlots disséminés présentent le même tableau clinique, fait de la combinaison des signes et symptomes suivants:

Température variable dans ses modes d'inscription et d'apparition;

Douleurs hépatiques spontanées plus souvent diffuses que localisées à prédominance hypochondrale droite mais s'étendant en arrière et remontant souvent jusqu'à la pointe de l'omoplate (la classique douleur en bretelle);

Douleurs hépatiques provoquées par l'ébranlement en masse;

Hypertrophie globale de toute la masse hépatique (bien qu'il puisse exister des hypertrophies portant sur le lobe droit ou sur le lobe gauche);

Réaction diaphragmatique et pleuro-pulmonaire droite que l'examen radiologique met toujours en évidence: surélévation et hypocinésie du diaphragme, minimes degrés de fermeture des sinus, opacité discrète de la base pulmonaire qui correspond à une obscurité respiratoire;

Signes généraux d'infection, leucocytose polynuclée moyenne, augmentation considérable de la V.S.H.;

Les tests de l'insuffisance des fonctions du foie sont négatifs.

Chez certains malades le faisceau syndromique n'est pas harmonieusement combiné. La prédominance marquée d'un symptome ou d'un signe à permis de décrire l'amibiase hépatique à type pseudo-palustre, pseudo-dothienentérique, à type respiratoire, tumoral, etc.

Dans ces occurrences le diagnostic peut être parfois difficile.

L'amibiase hépatique d'accompagnement est évidemment plus facile à reconnaître que l'amibiase hépatique cliniquement primitive.

L'examen coprologique est sans grand intérêt.

Les appellations de point de côté hépatique, congestion hépatique, Indian liver, Tropical liver, foie tropical, hépatite, hépatite présuppurative, traduisent un effort de classification des atteintes hépatiques amibiennes de la bénignité vers la gravité.

Les premiers de ces termes s'appliquent aux cas où les symptomes fonctionnels et les signes physiques sont modérés. Les cas d'apparence plus graves sont volontiers nommés hépatite pré-suppurative de Rogers, hépatite selon Fontan. L'expression d'hépatite pré-suppurative très évocatrice de l'état de nécrose parcellaire, encore que le mot d'hépatite s'applique mal aux désordres amibiens, n'a cependant de sens que clinique, elle ne saurait en rien préjuger de l'état anatomique. Le clinicien l'emploie pour désigner une amibiase hépatique dont la gravité lui paraît moyenne et dont il pense qu'elle pourra guérir par un simple traitement médical amoebicide.

Sans proscrire absolument ce terme que la médecine tropicale utilise depuis très longtemps, il vaut mieux ne pas employer un vocable qui engage le diagnostic lésionnel nullement établi.

Ces considérations nous permettent, abandonnant les traditionnelles et arbitraires appellations classiques, de considérer très simplement deux seules éventualités cliniques (il ne saurait en fait y en avoir d'autres):

*a*) L'amibiase hépatique aiguë, dont il est impossible par l'examen clinique de préjuger du type lésionnel;

*b*) L'abcès du foie cliniquement évident et indiscutable.

Dans la première éventualité, quelle que soit l'importance des symptomes fonctionnels et des signes physiques, le traitement médical spécifique sera entrepris et son action contrôlée tous les jours, tout au moins trois fois par semaine, par la mesure de la V.S.H.

Au cas de nécrose hépatique en îlots disséminés ou confluents en abcès de minime volume, les chiffres de la vitesse sédimentaire quotidiennement mesurée tendront vers la normale et l'atteindront plus ou moins vite à la disparition de la nécrose.

Au cas de nécrose collectée, abcès de notable volume, quelles que puissent être les améliorations apportées aux symptomes fonctionnels et aux signes physiques et à l'état général du malade, la V.S.H. toujours augmentée traduira l'existence du séquestre trop volumineux pour avoir pu être rapidement supprimé par les réactions tissulaires de régénération. La médication amoebicide n'aura été que la première étape d'un traitement dont la deuxième sera nécessairement d'élimination chirurgicale de l'abcès stérilisé.

La ponction sera alors pratiquée, exploratrice au vrai sens du terme, elle cherchera et trouvera à coup sûr la collection existante et ne sera que le premier temps d'un acte chirurgical exécuté à froid.

Nous utilisons cette technique depuis une dizaine d'années, sans jamais l'avoir vue une seule fois en défaut. Ce procédé simple nous a toujours permis de détecter les abcès du foie physiquement asymptomatiques.

Dans les abcès du foie cliniquement évidents et indiscutables, l'attitude thérapeutique est simple, c'est affaire de sens clinique sinon de bon sens. Si l'ouverture de l'abcès est une éventualité menaçante et prochaine: fluctuation sous une paroi amincie, imminence d'ouverture dans un viscère voisin, il faut intervenir. La destruction thérapeutique des amibes ne saurait être immédiate, l'action de l'amoebicide, si importante et si rapide qu'elle soit, ne peut enrayer instantanément la migration dangereuse.

Le chirurgien fixera la tactique opératoire et évacuera la collection. Le traitement médical par amoebicide sera concuramment institué et poursuivi jusqu'à la posologie totale légitime.

Si la collection ne présente pas les dangers d'ouverture spontanée et de migration immédiate, le traitement médical sera entrepris par

des amoebicides diffusibles habituels et l'intervention chirurgicale retardée sera pratiquée à froid.

Que l'intervention soit immédiate ou retardée, la mesure de la V.S.H. permettra le contrôle de son efficacité.

Les seuls amoebicides à utiliser dans l'amibiase hépatique sont les «amoebicides diffusibles» capables d'atteindre l'amibe par voie sanguine dans tous ses habitats tissulaires. Les oxy-quinoléines iodiques et certains arsenicaux sont évidemment sans action. Seules, l'émétine, la conessine ont une valeur incontestable. Les amino-4-quinoléines, les amino-9-acridines, que leur noyau hétérocyclique et leur stockage au niveau du foie pouvaient faire créditer d'un pouvoir amoebicide important, ont été utilisés avec succès. Nous avons employé sur plus de 600 malades des médications iodiques diffusibles tels que l'iodopropanol sulfonate de soude et l'iodhydrate d'hexamethylène tetramine ethanol avec un authentique succès.

## ASPECTS CLINIQUES ET NOSOGRAPHIE DE L'AMIBIASE RESPIRATOIRE

C'est toujours à partir de l'amibiase hépatique que s'ordonne l'amibiase respiratoire par des mécanismes simples commandés par l'anatomie thoraco-abdominale et la physio-pathologie amibienne:

Migration d'un abcès amibien de la convexité du foie qui aboutit, selon l'état pleural et diaphragmatique sus-jacent, à une vomique, un abcès pulmonaire, un empyème;

Extension de proche en proche à travers des adhérences hépato-phréno-pleurales d'un processus de nécrose hépatique amibienne en îlots disséminés qui réalise comme au niveau du foie une réaction congestive (congestion pulmonaire), une nécrose parcellaire (pneumonite), une nécrose confluente (abcès);

Défaillance du filtre hépatique et débordement de sa fonction d'arrêt qui laisse passer l'amibe qu'arrête le poumon, filtre secondaire: amibiase pulmonaire d'apparence primitive, abcès, pneumonite.

Au niveau du poumon, comme au niveau du foie, l'expression anatomique de l'agression amibienne est fonction de l'importance de l'ensemencement et de la valeur des résistances de l'organisme. L'ensemencement massif des migrations d'abcès hépatique se soldant par une nécrose collectée, l'ensemencement plus discret des extensions de proche en proche ou des métastases pouvant être sanctionné aussi bien par la nécrose collectée de l'abcès que la nécrose en îlots de la pneumonite.

La conception de Deve en matière d'échinococcose du poumon doit être acceptée pour l'amibiase. Il n'est pas plus d'atteinte pulmonaire amibienne sans atteinte hépatique que de kyste hydatique du poumon sans kyste hydatique du foie.

Toujours, dans le tableau clinique de l'amibiase respiratoire l'atteinte hépatique pourra être mise en évidence, même dans ses expressions les plus mineures, par un examen bien conduit.

## Nosographie respiratoire amibienne.

Elle découle de tout ce qui précède. Les atteintes pleuro-pulmonaires amibiennes peuvent être divisées en:

Manifestations réactionnelles pleuro-pulmonaires de voisinage au cours de l'amibiase hépatique, qu'il s'agisse d'amibiase hépatique suppurée ou d'amibiase hépatique pré-suppurative;

Atteintes pleuro-pulmonaires trans-diaphragmatiques par migration d'une amibiase hépatique suppurée ou par extension de proche en proche d'un processus nécrotique parcellaire:

*a*) Fistule broncho-hépatique;
*b*) Abcès pulmonaire par extension d'un abcès du foie;
*c*) Empyème provenant d'un abcès hépatique;
*d*) Congestion pulmonaire;
*e*) Pneumonite;
*f*) Abcès.

Manifestations pulmonaires en apparence primitive:

*a*) Abcès cliniquement primitif du poumon (abcès hématogène);
*b*) Pneumopathie amibienne non suppurée (pneumonite).

Le diagnostic des diverses manifestations de l'amibiase respiratoire est plus ou moins difficile selon les circonstances cliniques.

Les réactions pleuro-pulmonaires de voisinage ne posent aucun problème. Elles font partie intégrante du tableau de l'amibiase hépatique.

Les atteintes pleuro-pulmonaires suppurées ou non suppurées sont très rapidement rapportées à leur origine, quand l'amibiase hépatique s'impose par ses signes et symptomes. Elles peuvent être méconnues quand les signes et symptomes moins nets de l'amibiase hépatique n'on pas été reconnus ou n'ont pas été retenus. L'évolution apportera alors, mais tardivement, sa contribution au diagnostic. Le traitement antibiotique systématiquement entrepris dans toute pneumopathie aiguë échoue et l'amibiase hépatique s'affirme peu à peu par ses signes et symptomes, qui ne pourront plus être méconnus. La rapidité d'action d'un traitement spécifique par l'émétine enlèvera tous les doutes.

*Reconnaître l'étiologie amibienne d'un syndrome pleuro-pulmonaire c'est avant tout faire le diagnostic de l'amibiase hépatique.*

*Quand un syndrome pleuro-pulmonaire siège à droite, s'accompagne d'un foie augmenté de volume plus ou moins douloureux spontanément, douloureux nettement à l'ébranlement, il y a lieu de penser fortement à une atteinte amibienne si le passé du malade est entaché d'amibiase colique et même s'il ne l'est pas.*

Aucun test de laboratoire n'a de valeur ni hématologique, ni biochimique, ni biologique.

Nous ne saurions développer ici le diagnostic différentiel de l'amibiase pleuro-pulmonaire, qui dans les formes abcédées est extrêmement étendu, celui de toutes les suppurations pulmonaires.

Le traitement de l'amibiase pleuro-pulmonaire est parfaitement assuré par l'émétine, amoebicide diffusible qui atteint l'amibe au niveau du parenchyme pulmonaire; la conessine peut être utilisée à son défaut.

Les amino-4-quinoléines et amino-9-acridines ont une action moins marquée sur les localisations pulmonaires que sur les localisations hépatiques eu égard à leur concentration moindre au niveau du poumon.

Nous avons utilisé les iodiques solubles (iodo-propanol, sulfonate de soude, iodo-hydrate d'héxamethylène tetramine ethenol) avec des succès comparables à ceux enregistrés dans le traitement de l'amibiase hépatique.

Les antibiotiques n'ont d'action que dans les formes suppurées, trainantes, longtemps méconnues et secondairement infectées.

## ASPECTS CLINIQUES ET NOSOGRAPHIE DE L'AMIBIASE EXTRA-COLO-HÉPATO-RESPIRATOIRE

Les nécroses parenchymateuses liées au développement de l'amibe dysentérique ne peuvent qu'être exceptionnelles hors du foie et de l'appareil respiratoire.

Deux mécanismes pathogéniques les commandent:

L'extension d'un processus nécrotique amibien par contiguïté et adhérences à partir du colon, du foie, de l'appareil respiratoire (amibiase péricardique, urinaire, splénique, cutanée ...);

La métastase amibienne par défaillance successive des deux filtres hépatique et pulmonaire (amibiase encéphalique, ganglionnaire, ostéo-articulaire ...).

Le chapitre des localisations extra-colo-hépato-respiratoires de l'amibiase est très vaste et occupe en littérature médicale une place qui ne correspond en rien à son importance réelle. Il ne s'agit jamais que de manifestations aberrantes et très rarement rencontrées.

En dehors des amibiases viscérales par contiguité dont la nature amibienne est évidente, le diagnostic d'amibiase extra-colo-hépato-respiratoire ne doit être accepté qu'après une critique poussée qui fera intervenir l'examen histologique, la parasitologie et l'épreuve thérapeutique spécifique. Cette dernière à accepter comme déterminante seulement dans ses résultats quasi-spectaculaires.

Il va sans dire qu'il ne saurait être question ici de développer ni même d'ébaucher la description de toutes les manifestations morbides extra-colo-hépato-respiratoires attribuées à l'amibe et d'en faire une étude critique. Nous énumérerons simplement les localisations qui en ont été rapportées.

## Les amibiases viscérales par contiguité.

### *Les péricardites amibiennes.*

L'ouverture d'un abcès amibien du foie dans le péricarde a été signalée par de nombreux auteurs: R. Shaw, R. Elsdon-Dew, etc. De Cyr, Stitt, sur 159 cas de perforation d'abcès du foie, notent 31 migrations abdominales, 59 pulmonaires, 31 pleurales, 1 péricardique.

Il s'agit le plus souvent d'abcès du lobe gauche. L'exitus sanctionne pratiquement toujours l'irruption péricardique.

Quelques cas, cependant, chirurgicalement et médicalement traités, ont pu guérir (R. Coirault et coll.).

### *L'amibiase splénique.*

Elle n'est pas absolument exceptionnelle et nous avons eu nous-même l'occasion d'en rapporter trois cas (F. Blanc et P. Navarranne). Son expression clinique est l'abcès de la rate parfaitement sensible à la thérapeutique émétinienne (Frank, Manson-Bahr, Huard, etc.). Il ne s'agit pas la plupart du temps d'amibiase métastique, mais de communication directe colo-splénique ou hépato-splénique.

### *L'amibiase génito-urinaire.*

Les seules manifestations incontestables de l'amibiase génito-urinaire, celles qui répondent à la notion de nécrose amibienne sont extrèmement rares.

Une communication hépato-rénale peut se solder par un abcès amibien du rein. L'existence de fistules colo-vésicales a pu être signalée (Baelz, 1883).

On ne saurait tenir les néphrites qui surviennent parfois chez les amibiens comme l'expression d'une localisation rénale de l'amibe (étude critique de Watson, 1945).

Les uréthrites apparaissant chez les amibiens rappellent les écoulements observés dans les dysenteries bacillaires et la fréquence des associations amoebo-bacillaires doit rendre singulièrement circonspect dans l'affirmation de la nature amibienne d'un écoulement.

Les localisations au niveau de la verge qui ont été publiées paraissent le fait de contamination directe (Kouri, Bolanos, Fuentes, etc.) et sont à placer à côté des amibiases cutanées.

*L'amibiase cutanée.*

Localisation rare mais qui demande à être bien connue eu égard à sa gravité redoutable et aux erreurs de diagnostic qu'elle peut entrainer. Elle a été longuement étudiée et remarquablement par Touraine et Duperrat (1939).

L'amibiase cutanée par contiguïté, l'amibiase d'extension selon Touraine, comporte deux types d'accidents:

Les amibiases péri-anales compliquant certaines rectites amibiennes graves;

Les amibiases cutanées consécutives à l'ouverture d'un abcès amibien viscéral, essentiellement d'un abcès du foie, par ensemencement de l'empyème de nécessité ou de la plaie opératoire. Les aspects cliniques sont assez typiques. La plaie, après une courte phase erythémateuse, prend un aspect anthracoïde, puis sphacélique. La nécrose s'étend excentriquement et atteint en 15 à 20 jours une paume de main. L'extension est tantôt régulière, circulaire ou, au contraire, polycyclique. La nécrose s'élimine au centre, laissant une ulcération à vif. Cette ulcération fort douloureuse reste superficielle, ne détruit que la peau et le tissu cellulaire sous-cutané, ne s'accompagne d'aucune réaction ganglionnaire. L'état général s'altère très rapidement comme dans tous les processus gangréneux d'origine infectieuse. Avant les traitements émétiniens la mortalité était considérable.

À côté de cette amibiase de contiguïté sont à placer les amibiases cutanées d'inoculation. Les amibiases d'inoculation génitale, d'origine vénérienne, dont nous avons vu plusieurs cas, et les amibiases cutanées pures apparaissant en un point quelconque des téguments chez les sujets atteints d'amibiase colique.

L'aspect est comparable à celui des phagédénismes amibiens compliquant les ouvertures d'abcès et les amibes fourmillent dans les lésions (Enghan et Hetthaus).

Des abcès dermo-hypodermiques de lente évolution de petite taille, sans gravité, de nature amibienne ont été également signalés. Ils paraissent plus qu'exceptionnels (3 cas).

Ne pourrait-il s'agir d'abcès métastatiques?

*Les lésions inflammatoires de voisinage — Les amoebomes.*

C'est un chapitre assez confus.

Chez certains amibiens présentant des formes de longue durée, dysentériques, la palpation révèle des tumeurs coliques (Degorge et Houlels ... Lagnier, Huard, Montel; James, Gunn et Howard, Lee, etc.).

Ces tumeurs sont des cavités à parois fibreuses, développées à partir de l'hypertrophie des couches coliques, en communication avec la lumière intestinale.

Tumeurs inflammatoires banales où la présence d'amibes peut être constatée. La thérapeutique émétinienne est susceptible d'action immédiate et permet la correction du diagnostic de néoplasie facilement envisagé.

Nous avons eu à connaître, récemment, d'un malade amputé du sigmoïde qui n'était qu'un amibien (autochtone), comme le révélèrent l'examen histologique de la tumeur et le réveil dramatique post-opératoire de l'amibiase colique méconnue.

Les amibiases viscérales par métastase.

À côté des abcès pulmonaires, cliniquement primitifs, hématogènes, les abcès du cerveau sont les seules localisations métastatiques incontestables de l'amibiase, toutes les autres nous apparaissant des plus discutables.

*L'amibiase encéphalique.*

C'est une localisation très rare dont il a été publiée à peine une soixantaine de cas (Morehead, Legrand, Izar, Huard, etc.). Il s'agit presque toujours de malade ayant présenté une atteinte hépatique puis pulmonaire grave et la défaillance conjointe des deux filtres a permis par voie sanguine l'embol amibien au niveau de l'encéphale et l'action nécrotique consécutive.

Le diagnostic est le plus souvent le fait d'une vérification nécropsique. Dans une observation de Collard et Kendal le traitement émétinien s'avéra rapidement efficace.

*L'amibiase ostéo-articulaire.*

Hors l'observation toujours citée de Kofoid et Sweezi qui mentionne la présence d'amibes en certaines zones nécrosées de la trame osseuse, il n'est rien à retenir de ce chapitre.

Le rhumatisme peut parfaitement se rencontrer chez des amibiens eu égard à la fréquence des associations mixtes. Il est singulièrement difficile d'affirmer outre-mer qu'un bacille dysentérique ne représente pas la cause déterminante d'une colite amibienne.

It s'agit là plus ou moins directement de manifestations allergiques, inflammatoires, nullement d'une action directe de l'amibe elle-même.

*L'amibiase lymphatique.*

Elle a donné lieu à beaucoup de discussions et à mon sens bien gratuitement car l'anatomo-pathologie ne montre pas de retentissement ganglionnaire dans les atteintes coliques les plus marquées, pas plus dans les rares cas d'amibiase cutanée bien observés.

Le bubon amibien dont il a été pas mal écrit n'est qu'une lymphogranulomatose bénigne méconnue. L'action favorable de l'émétine sur la poradénolymphite fistulisée a pu faire admettre l'étiologie amibienne (Ravaut, Boulin et Rabaut).

Les adénopathies iliaques (Montel) sont peut-être à ranger dans la même rubrique.

# SOME RESULTS OF SEVERE AMOEBIASIS

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During the past 70 years an extensive literature dealing with the clinical aspects of amoebiasis has grown up. Two trends are apparent. On the one hand there are the descriptions of conditions in which there can be no doubt that the amoeba is an aetiological agent. On the other, there is a tendency to widen the symptomatology of this disease to include syndromes remote from the gut and liver in patients who happen to harbour *Entamoeba histolytica*. The latter trend is to be deprecated for obvious reasons. The clinical picture of amoebiasis has been very fully covered by papers of the first category and I can certainly add nothing new, but it seems worthwhile to consider some less common aspects of the disease which can lead to severe illness, prolonged disability or even death. These conditions are still seen with some frequency in areas where amoebic dysentery is endemic or when epidemics occur.

Between 2 and 3 thousand cases of amoebiasis are seen annually in one hospital in Durban, South Africa. They come mainly from the local African population, many of whom live in slum conditions on an inadequate diet and with primitive or no sanitation. The majority of these patients present with acute amoebic dysentery which responds well to standard methods of treatment, but in a number of cases the

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disease is unusually severe and complicated cases are frequently seen. I shall discuss three of these complications: (1) peritonitis, (2) post-dysenteric colitis, and (3) rectal stricture. My purpose is to emphasize some of the factors in diagnosis and treatment which our experience over the last 12 years has led us to regard as important if mortality and morbidity are to be kept minimal.

## GENERAL PERITONITIS, SECONDARY TO INTESTINAL AMOEBIASIS

Two types can be distinguished: The first is very rare and occurs when an amoebic ulcer perforates in a patient whose dysentery is only of moderate severity, or in a patient whose symptoms have already been controlled by treatment. Signs of peritonitis appear with dramatic suddenness and the case presents as a classical «acute abdomen». Immediate surgery is indicated as the gut surrounding the ulcer is likely to be sufficiently normal to allow of closure of the perforation.

The second type and much the commoner is a complication of severe amoebic dysentery in which the whole colon is grossly diseased. The gut has aptly been likened to wet blotting paper (Rogers, 1922) and it appears to become so permeable that infection may reach the peritoneal cavity without actual rupture of an ulcer, but frank perforation may also occur. It is this second type that I wish to discuss further.

The clinical picture is not that of the classical «acute abdomen», but rather that of gradual distension of the belly with moderate rigidity followed by ileus. Despite the ileus and increasing distension, diarrhoea may continue because of the outpouring of exudate from the grossly diseased colon. Bowel sounds are absent and dehydration and loss of electrolytes soon become prominent features. The presence of amoebiasis is easily confirmed by stool examination and or the careful introduction of a proctoscope when massive rectal ulceration will be seen. A sample of the exudate or slough covering the ulcers will contain myriads of haematophagous trophozoites of *E. histolytica*.

In our experience the important points in treatment are:

(1) To avoid surgery;

(2) Control of the amoebae and of secondary infection;

(3) To relieve distension and supply water and electrolytes.

I have seen the results of a variety of surgical measures, ranging from attempts to repair perforated ulcers, to drainage, ileostomy and even colectomy. Repair of a perforation is impracticable because it may not be found and even if it is, the surrounding gut is so friable that the hole is usually enlarged rather than closed. The other techniques confer no benefit and the end result is that a desperately ill patient bears the added burden of recovering from an unnecessary operation (Cope, 1920; Barker, 1958). Treatment is therefore conservative.

We feel that a tissue amoebicide is essential and we have used emetine. Tetracycline is given intravenously for control of bacterial infection and for its probably related control of intestinal amoebae. The abdominal distension must be countered by continuous or intermittent suction and fluid and electrolyte balance carefully maintained.

In our experience this treatment yields the best results, but though it has reduced mortality considerably, this is still high, probably about 20 % in the severer cases. Convalescence is long and may be complicated by post-dysenteric colitis.

## POST-DYSENTERIC COLITIS

This clinical condition has been well described by Stewart (1950). In our experience this complication is usually caused by a severe attack of amoebic dysentery. It is characterised by persistent diarrhoea and increasing wasting and debility of the patient. Blood and mucus often persist in the stools, but amoebae can no longer be found. At sigmoidoscopy the mucosa is red and oedematous and shallow ulceration is seen. Although there is less tendency for ulcers to be covered by exudate, the picture is not easily distinguished from active amoebiasis. Symptoms persist for weeks and even months, but in the majority of case full recovery eventually takes place. Occasionally it may cause death.

Repeated courses of amoebicides are of no value, neither do antibiotics seem to influence the course of this condition. We have tried steroids without convincing evidence of benefit. Treatment must be symptomatic and supportive, including blood tranfusion as a normocytic, normochromic anemia which does not respond to haematinics, is frequently present. The aim of treatment is to maintain the patient until spontaneous recovery takes place.

## RECTAL STRICTURE

In 1918 Lasnier first established that *E. histolytica* might cause granuloma formation in the gut and there is an extensive literature on amoeboma.

Rectal stricture *per se* has received less attention, but has been well described (Hillemand *et al.*, 1939; Ochsner and De Bakey, 1942; Hawe, 1945). Kark (1958) considers that amoebiasis is the commonest cause of rectal stricture among Durban Africans. The belief that these strictures are amoebic in origin is based on their association with proved amoebic dysentery, the finding of amoebae in biopsy specimens, the absence of other demonstrable causes, and their response to treatment.

During a 3 year period, 32 cases were seen in one unit and the following observations are based on analysis of this series.

*Incidence.* — This is very difficult to assess as the finding of strictures depends largely on regular sigmoidoscopy. The majority of patients suffering from this condition do not have symptoms directly referable to the stricture, but rather to the dysentery and unless sigmoidoscopy is done the presence of a stricture may well be overlooked.

*Appearance.* — In the early stages there is a localised annular area of granulation tissue, deep red in colour and studded with typical amoebic ulcers, from which trophozoites of *E. histolytica* can easily be recovered. This is usually situated 5-10 cm from the anus. Biopsy shows granulation tissue and amoebae can be identified. Specific treatment results in the rapid disappearance of amoebae and biopsy at this stage yields tissue which is histologically non-specific. Under treatment ulcers heal and the mucosa becomes more normal in appearance, but the stricture may contract until the lumen is reduced to a diameter as small as 1-2 cm.

Sometimes the appearance is that of a diaphragm-like structure covered by normal mucosa with a small centrally or slightly eccentrically placed aperture.

*Symptoms and course.* — In most cases stricture follows severe dysentery, but the duration of symptoms does not seem to be a factor in its production. As has been said, the stricture *per se* rarely causes

symptoms, but occasionally there may be a complaint of pain on defaecation. Only very rarely do symptoms of obstruction occur.

In a few cases (4 out of 32) the stricture resolved rapidly (within 1 month) on treatment. Much more commonly narrowing of the gut persisted for many months (in one case 14 months), but eventually in those patients which could be followed the rectum returned to normal. In many cases the patients ceased to attend hospital because they were symptom-free and none of them returned with symptoms referable to their strictures, although narrowing of the rectum had still been present at their last examination.

*Treatment.* — It has been shown that adequate treatment of the amoebic dysentery usually results in the eventual disappearance of the stricture, and symptoms are seldom prominent. For this reason I believe that these cases should be treated conservatively and more active direct surgical methods reserved for those few cases in which symptoms are incapacitating or in which obstruction is a real danger. It is my impression that in two patients on whom colostomy was done, the rectal stricture tended to become fibrous and permanent. It may be that the daily passage of faeces through the narrowed rectum acts as an «auto-dilator» and tends to keep the lumen patent.

## CONCLUSIONS

I have discussed three of the many complications of intestinal amoebiasis which may be encountered when large numbers of acute cases of amoebic dysentery are seen. They illustrate serious results which may follow fulminating infections and emphasise the necessity of early treatment with an effective amoebicide, and in this respect I do not think emetine has been bettered. Antibiotics for control of bacterial infection and indirectly of the amoebae and supportive treatment are of prime importance. Surgery in general should be avoided, except in a few fairly well-defined instances when it may indeed be life-saving.

## BIBLIOGRAPHY

Barker, E. M. — *S. Afr. Med. J.*, **32**: 634, 1958.
Cope, Z. — *Surgical Aspects of Dysentery*. Oxford Univ. Press. London. 1920.
Hawe, P. — *Surg. Gynec. Obstet.*, **81**: 387, 1945.
Hillemand, P., Bensaúde, A., and Bru, P. — *Bull. Soc. Med. Hop. Paris.*, **55**: 537, 1939.
Kark, A. E. — Personal communication. 1958.
Lasnier, E. P. — *An. Fac. Med. Montevideo*, **3**: 809, 1918. Quoted by Spicknall, C. G., and Peirce, E. C. — *New Engl. J. Med.*, **25**: 1055, 1954.
Ochsner, A., and De Bakey, M. — *Int. Clin.*, **1**: 68, 1942.
Rogers, L. — *Lancet*, **1**: 463, 1922.
Stewart, G. T. — *Brit. Med. J.*, **1**: 405, 1950.

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# «11925 C» IN THE TREATMENT OF AMOEBIASIS AND OF SOME RELATED DISORDERS

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The treatment of amoebiasis, so important in our countries, is a problem of perennial interest, arousing every now and then hopes of permanent cure. In spite of the progress achieved by the introduction of emetine and its derivatives, of drugs of the quinoline series, of the arsenicals, of the antibiotics, and recently of the acridine compounds, I have lived long enough to see hopes in each case disappointed, as preliminary sanguine claims appeared less justified with sufficiently prolonged follow-ups. We had, in each case, to fall back on tedious repetition, alternation, and combination of the available drugs to procure to the patient an acceptable *modus vivendi*.

A new preparation was recently launched under the laboratory number of 11925 C. This is 4,7,phenanthrolene-5,6,quinone, which, according to its manufacturers, is bettered *in vitro* only by emetine as a direct amebicide, and possesses bacteriostatic properties against the common intestinal pathogens, *B. coli, Shigella, Sonne, Streptococcus faecalis* and *Staphylococcus aureus*, superior to those of vioform.

It is stated to be readily absorbed and to appear in the blood, urine, and bile in amoebicidal form. Its therapeutic margin is extremely wide (0.5 g per kilo bodyweight in the rat was not toxic). It is issued in 50 mg tablets.

## MATERIAL AND METHODS

The drug was tried in 70 patients suffering from acute or chronic amoebiasis, and in 20 patients with non amoebic colitis. All the patients lived in town, and were advised not to eat any raw vegetables or salads during the period of observation in order to minimize the risk of reinfection.

The drug was given after a meal, 0.100 g 4 times daily for 10 days. After completion of the course, the stools were re-examined weekly by direct smears, staining with iodine, and in some cases culture on 3 successive occasions. In those cases that relapsed a similar course was given. We could perform sigmoidoscopy only in 12 cases.

## AMOEBIC DYSENTERY AND COLITIS

Only cases with symptoms were treated. So called «cyst passers» were not included. Vegetative forms of *E. histolytica* were found in the stools in 19 cases, both cysts and vegetative forms in 19 cases, cysts alone in 32 cases. In addition, 35 harboured other parasites:

| | |
|---|---|
| *Trichomonas* ... ... ... ... ... | 8 |
| *Blastocystis hominis* ... ... ... ... | 15 |
| *Giardia lamblia* ... ... ... ... ... | 7 |
| *Chilomastix mesnili* ... ... ... ... | 5 |

Hepatitis was present in 6 cases. These findings are summarized in Table 1.

## EFFECT ON *E. HISTOLYTICA*

The stools were cleared of the vegetative forms after the first course in 6 out of 19 cases (31.6 %), of cysts in 12 out of 32 (37.5 %) and of both trophozoites and cysts in 5 out of 19 (26.2 %).

Of these, roughly one third relapsed within 2 months. They were cured after a second course.

TABLE 1

| | Total number | Improved with pos. stools | Results of first course of treatment | | | Abandoned | Second course | | | Needed third course |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cured | Days after which improvement appeared | Microsc. failure | | Number of cases | Days after which relapse occurred | Cured | |
| *E. histolytica*, vegetative | 19 | — | 6 (31.6 %) | — | 13 | 1* | 12 | — | 11 | 1 |
| *E. histolytica*, cysts | 32 | — | 12 (37.5 %) | — | 20 | 5 | 15 | — | 14 | 1 |
| *E. histolytica*, cysts + vegetative | 19 | — | 5 (26.2 %) | — | 14 | 4 | 10 | — | 8 | 2 |
| *Trichomonas* | 8 | — | 6 | — | 2 | — | — | — | 2 | — |
| *Giardia* | 7 | — | 6 | — | 1 | — | — | — | 1 | — |
| *Chilomastix* | 5 | — | 4 | — | 1 | — | — | — | 1 | — |
| *Blastocystis* | 15 | — | 0 | — | 15 | — | — | — | 0 | — |
| Acute dysentery | 4 | 2 | 1 | 2.3 | 2 | 1* | 2 | 10 d | 2 | 0 |
| Chronic am. colitis | 66 | 35 | 22 | 4.0 | 45 | 9 | 35 | 12 d | 31 | 4 |
| Hepatitis | 6 | 4 | 2 | 3.5 | 4 | 0 | 3 | 12 d | 4 | 0 |

* Had to take emetine.

Ten of the failures abandoned the treatment for various reasons. The remaining except 4 were cured after a second course. These 4 were cured after a third course.

We consider as a cure the absence of relapse to the date of writing this paper, i.e., for periods varying from 2 to 4 months.

## OTHER PARASITES

*Giardia* was found in 7 cases; 6 were cured after a single course, and the remaining after a second course; *Blastocystis hominis* (15 cases) was not affected. *Chilomastix mesnili,* 5 cases; 4 cured after one course, the fifth unaffected by 2 courses; *Trichomonas.* 8 cases, of which 6 were cured after one course, 1 after a second course, and 1 unaffected.

## EFFECT ON THE SYMPTOMS

*Acute cases* (4 cases). — Improvement appeared twice after 2 days, and once after 3 days. One case did not improve and had to be put on emetine hydrochloride. Of the 3 that improved, 2 relapsed and were cured after a second course.

*Chronic cases* (66 cases). — In chronic cases suffering from such vague symptoms as mild diarrhea, diarrhea alternating with constipation, flatulence, vague abdominal pain, or headache, improvement appeared later, as an average after 4 days (limits 2-8 days).

The symptoms were cured permanently in 22 cases after a first course. In 35 other cases, they disappeared but re-appeared 2 days after cessation of the drug; 9 abandoned. In the remaining 35 cases, a second course brought permanent relief in 31; the remaining 4 needed a third course. In those cases that were ameliorated, the thickened colon improved with remarkable rapidity.

*Hepatitis* was present in 6 cases as judged by an enlarged, tender liver reaching at least two fingers below the costal margin, tenderness and pain over the lower right intercostal spaces, and in 3 cases by a leucocytosis of 10,200, 12,300, and 13,400. In 2 cases

examined radiographically, the diaphragm was raised and immobile. No liver biopsies were performed.

One of these patients was a lady with a chronic relapsing dysentery, who had had several courses of emetine, and whom we had previously treated with enterovioform, terramycin and aralen with only temporary results. She was harbouring vegetative amoebae and cysts, and had no free HCl in her gastric juice. She had to take continuously Ac Hydrochl. Dil. with meals to keep down her diarrhea.

After the first course, the liver was no longer palpable, all tenderness had disappeared, and she passed only one formed stool a day in spite of having stopped the hydrochloric acid.

Two weeks later, the diarrhea, the tenesmus, and hepatic enlargement recurred, and *E. histolytica* were found in the stools. After a second course, she was permanently relieved. However, owing to her previous experience, she insisted on taking two other courses of the drug and it was difficult to persuade her not to make it a lifelong medication.

## SIDE EFFECTS

The drug is slightly laxative; this was most convenient to the chronic constipated cases, many of whom wanted to continue taking it for that purpose. It had to be withdrawn on account of diarrhea in 2 cases. Vomiting was complained of by one young lady who was suffering from considerable nausea before the treatment. She had to abandon taking it.

One patient complained of passing a dark urine. As she was away at the time, she was advised to have her urine examined for blood. It proved negative. The colour was most probably due to oxidation products of the drug.

Three of our patients were pregnant. Two were thyrotoxic. They tolerated the drug very well.

*Sigmoidoscopy* was performed in 12 cases before, and a week after, completion of the first course of treatment.

Of these 12, 9 were clinical and microscopic cures. Eight of them showed complete healing. The ninth still had a few ulcers and

relapsed microscopically after a week. The remaining 3 could not be re-examined after the second course.

## EFFCT OF «11925 C» ON CHRONIC NON-SPECIFIC COLITIS

Twenty cases of chronic colitis in whom no parasites could be discovered on repeated stool examination were submitted to the treatment.

The symptoms were the following:

TABLE 2

| Symptoms | Number of cases out of 20 | Improved |
|---|---|---|
| Constipation, flatulence, mild colic | 14 | 10 |
| Diarrhea | 3 | 0 |
| Constipation alternating with diarrhea | 3 | 1 |
| Tender thickened iliac colon in addition | 16 | 12 |
| Tender caecum with gurgling | 7 | 4 |
| Tender, not enlarged liver | 5 | 2 |

It is obvious that much improvement was observed in the chronically constipated patients, and none in the diarrheic. The effect was not purely due to laxation, as (*a*) in our experience laxatives aggravate the colic and flatulence, and (*b*) the tenderness over the colon or coecum disappeared. It was most probably due to the bactericidal effect of the drug. However, as no sigmoidoscopic examinations were performed, it may well be that, at least, some of these may have been cases of chronic amoebiasis. In our experience, a third may have been expected to be such cases.

## COMMENT

1. It is obvious that a single course of 10 days' treatment is quite inadequate to cure amoebiasis, in spite of the great clinical improvement that is observed even when the stools are not cleared.

This great improvement occurred although no adjuvant, dietetic or medicinal was prescribed, except the recommendation to avoid raw vegetables. It was probably the result of the bactericidal effect of the drug combined with its mild laxative properties. However, a second course brings about a cure in a remarkably high percentage of cases. Of the 60 who pursued the treatment, 23 were cured after a first course (38 %), a further 33 (55 %) were cured after a second course, and 4 (7 %) were cured after a third course. The latter have only been followed for 2 months and we cannot foresee whether there will not be late relapses.

These results are extremely encouraging. It remains for the future to find out whether combinations of this compound with other drugs can shorten the treatment, obviate the necessity of repetition of the course, or diminish the number of failures.

2. In non-amoebic cases, chronic colitis associated with constipation may be expected to improve in about 70 % of the cases with disappearance of the thickening and tenderness of the colon.

# ANTIBIOTIC TREATMENT OF AMEBIASIS

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With the introduction of antibiotics into the field of therapy, there was an immediate attempt to evaluate their effectiveness in all forms of amebiasis from acute fulminating amebic dysentery to the symptomless infection with *Entamoeba histolytica*. The value of a large number of antibiotics in the treatment of amebiasis has now been studied extensively in many different areas of the world.

As early as 1945 Hargreaves reported that 57 patients with resistant amebiasis showed marked improvement when treated with penicillin. He found that the temperature in these patients usually became normal within 24 hours after treatment was started. The stools in most instances became normal within 48 hours, but the amebae were still abundant in the stools.

Shaffer and Frye (1947) showed that penicillin and streptomycin, either alone or combined, in fairly high concentrations, when added to cultures of *E. histolytica* had little if any effect on the growth and multiplication of the amebae. These antibiotics did inhibit growth of certain bacteria in the cultures and in some cases the ameba growth in penicillin-streptomycin treated cultures was improved.

In 1949 Armstrong, Wilmot and Elsdon-Dew observed partial or complete healing of amebic ulcers following treatment with penicillin and sulfasuxidine.

Clinical successes using aureomycin in the treatment of amebiasis was first reported by McVay and his associates in 1949. This group of workers reported 19 of 23 cases parasitologically negative after

treatment with 2 g daily for 7 days. Most *et al.* (1950) obtained better than 90 % sucess in 33 cases, most of which were mild or asymptomatic amebiasis, when treated with 2 g of aureomycin daily.

In 1950 Armstrong, Wilmot and Elsdon-Dew reported the use of aureomycin in acute amebic dysentery. They obtained 94 % success in 50 patients showing ulceration by sigmoidoscopy. In only one of the 50 patients was there complete failure of response from both the clinical and parasitological standpoints. Two parasitologic failures were clinically improved.

Oxytetracycline was developed in 1950. During the same year Most found this broad spectrum antibiotic highly effective in eradicating *E. histolytica* from the intestinal tract of man. In 1951 Tobie *et al.* reported the results of mass treatment trials with oxytetracycline in a state mental institution. They reported oxytetracycline 100 % effective in eliminating *E. histolytica* in this group of asymptomatic carriers, on the basis of 6 months follow-up.

Killough and Magill (1951) reported results of treatment in 7 cases of acute amebic dysentery with oxytetracycline. All of the patients complained of generalized abdominal cramps and bloody diarrhea with mucus. Stools were positive for *E. histolytica*. Proctoscopic examination revealed various degrees of mucosal ulceration, with inflammation, and edema. Scrapings of these ulcers showed numerous motile *E. histolytica* trophozoites. The dose of oxytetracycline administered for the first 3 days was 150 mg per kilogram per day, after which it was reduced to 75 mg per kilogram per day for the remainder of the treatment period of 14 days. In all patients there was disappearance of bloody diarrhea, tenesmus and other dysenteric symptoms within 2 to 6 days. Stools and proctoscopic specimens became negative for amebae after 2 days in 4 patients and after 8 days in the remaining patients. In 1 patient an amebic hepatic abscess developed during oxytetracycline therapy.

The most extensive evaluation of the antibiotics in the treatment of acute amebic dysentery has been reported by Elsdon-Dew and his collaborators in Durban, South Africa. As early as 1950 Armstrong, Wilmot and Elsdon-Dew reported results with a number of amebacides, developing a standard method for assessment of results. They were so impressed by the results obtained by Hargreaves that they

decided to repeat his multiple therapy, using penicillin and sulfasuxidine. The results obtained with this combination were very good. Penicillin alone and sulfasuxidine alone, though having no apparent direct antiamebic action, were capable of curing some 60 % of cases when combined. The results with penicillin were surprising, in view of the fact that it had no effect on *E. histolytica* in culture.

Their results with two broad-spectrum antibiotics, oxytetracycline and chlortetracycline, were excellent. Not only did these antibiotics show a success rate of over 90 %, but cleared both ulcers and amebae with a rapidity not previously encountered. In the first 10 days of treatment 82 % of cases treated with oxytetracycline were clear of ulcers and amebae. These two broad-spectrum antibiotics gave similar results; both showed a rate of cure far and away beyond any single therapy they had used.

In 1952 Frye, Brooke and Weinstein reported the results obtained with three broad-spectrum antibiotics in the treatment of acute amebic dysentery. They compared the results obtained with oxytetracycline, chlortetracycline and chloramphenicol in patients with lesions demonstrable on sigmoidoscopic examination. Success depended upon the disappearance of the lesions and clearing of *E. histolytica* from the intestinal tract. All patients were followed for a minimum of 6 weeks. Oxytetracycline was found to be most effective, with only 1 failure in 40 patients treated. There were 12 failures in a group of 41 treated with aureomycin and 18 failures in 39 treated with chloramphenicol.

Martin *et al.* (1953) reported the final results of this study done in South Korea. In addition to the studies done with the broad-spectrum antibiotics a comparison was made with some of the standard amebacides. The results of this study in 330 acute cases of amebic dysentery are shown in Table 1. Selection of cases was rigid, and the 6 weeks follow-up was done in the hospital.

The standard amebacides used individually were inadequate therapeutic agents in this group of patients. Combinations of standard amebacides proved to be much more effective. In 23 cases a combination of emetine, carbarsone and chiniofon gave good clinical response. None had recurrence of clinical symptoms, but 4 had reappearance of *E. histolytica* in the stools at the end of 6 weeks.

Oxytetracycline proved to be the most effective of the antibiotics. A total of 104 patients were treated with this antibiotic and all

responded clinically. Six relapsed, however; 3 early in the follow-up period and 3 later. One of these 6, although his intestinal lesions healed, developed clinical amebic hepatitis.

Aureomycin also produced a good initial response in all but 3 of the 41 cases treated. At the end of the 6 weeks follow-up 7 of the remaining 38 patients had *E. histolytica* in the stools, and 4 of these had symptoms.

TABLE 1 — *Comparison of therapeutic response in 330 cases of acute amebic dysentery treated with standard amebacides and three broad-spectrum antibiotics assessed at end of 6 weeks*

| Therapeutic agent | Number treated | Therapeutic response | |
|---|---|---|---|
| | | Success | Failure |
| *Standard amebacides* | | | |
| Emetine | 22 | 10 | 12 |
| Carbarsone | 22 | 4 | 18 |
| Chiniofon | 24 | 9 | 15 |
| Chloroquine | 31 | 13 | 18 |
| Emetine-carbarsone and chiniofon combined | 23 | 19 | 4 |
| Milibis-aralen | 22 | 11 | 11 |
| *Broad spectrum antibiotics* | | | |
| Oxytetracycline | 104 | 98 | 6 |
| Chlortetracycline | 41 | 31 | 10 |
| Chloramphenicol | 41 | 11 | 30 |

Chloramphenicol proved to be ineffective. Only 11 of 41 patients treated responded satisfactorily, and most of the patients who were treatment failures had *E. histolytica* in their stools until they were re-treated with another drug.

In this study there were 66 patients followed without specific therapy. At the termination of the 6 weeks follow-up period 11 of the 66 patients had no clinical or parasitological evidence of amebic infection. These patients, by the standards used, would be counted as successfully treated, and in addition 5 others with stools positive for *E. histolytica* had recovered clinically. These observations prove

that spontaneous recovery does occur and must be given consideration in the evaluation of any specific therapy.

Wilmot (1956) reported on a comparative study of the treatment of 136 cases of acute amebic dysentery with chlortetracycline, oxytetracycline and tetracycline. All three of these broad spectrum antibiotics were efficacious and of equal value in the immediate control of symptoms, elimination of amebae and healing of ulcers.

Puromycin was used by Wilmot to treat 24 cases of acute amebic dysentery. He found that this antibiotic was efficacious in ridding patients of *E. histolytica,* but control of symptoms was slow and healing of ulcers delayed by comparison with tetracycline and its derivatives.

A preliminary report in 1953 of the use of fumagillin was made by McHardy *et al.* This drug was found by Balamuth (1953) to have the best amebacidal value in a series of amebacides tested *in vitro.* Sappenfield *et al.* (1955) treated over 350 carriers with fumagillin and a similar number of infected individuals with terramycin. The results of this study are recorded in Table 2. This study was done

TABLE 2 — *Treatment of asymptomatic amebiasis with oxytetracycline and fumagillin*

| Therapeutic agent | Number treated | Percent negative after three months follow-up |
|---|---|---|
| Oxytetracycline . . | 378 | 93.7 |
| Fumagillin . . . . . | 369 | 93.5 |

in a commercial plant in South Bend, Indiana. The large number of individuals infected with *E. histolytica* resulted from a water borne epidemic within the plant. None of the individuals treated had any gross evidence of clinical disease at the time of treatment. All were treated as ambulatory patients in the plant by a team of physicians and nurses. All were checked daily for any possible reaction to the antibiotics during and after treatment. Each patient was followed for a period of 3 to 4 months with repeated stool examinations of all patients

treated. It is of interest to note that the results were almost identical with these two antibiotics. In the 378 treated with oxytetracycline 93.7 % were negative at the end of the follow-up period and in 369 treated with fumagillin 93.5 % were negative.

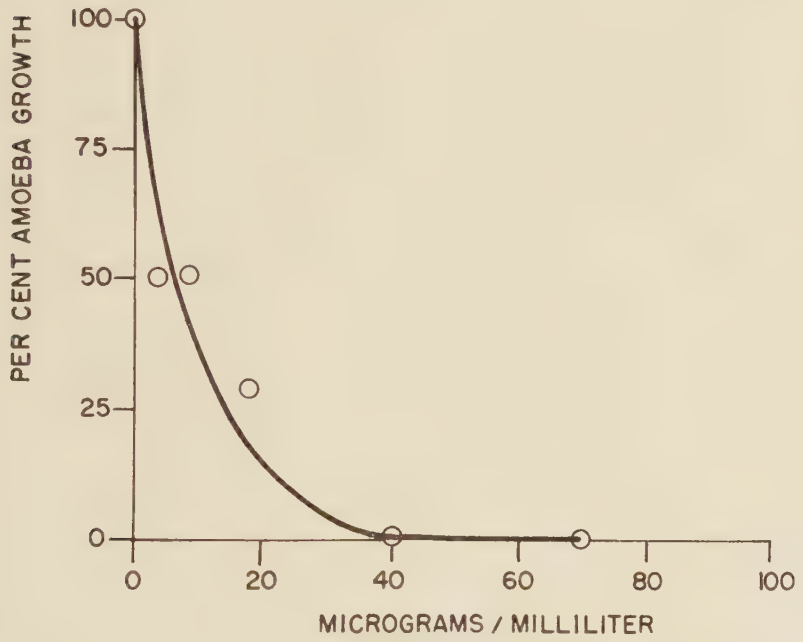
Several workers have found fumagillin effective in the treatment of acute amebic dysentery. Elsdon-Dew (1953) treated 7 cases with 40 mg of fumagillin per day. The results were disappointing in these cases. Three had to be removed from treatment because of dangerous clinical deterioration and the remaining 4 all showed ulceration and parasites at the completion of treatment.

Numerous other antibiotics have been evaluated in the treatment of amebiasis. In 1953 McCowen *et al.* reported that erythromycin (ilotycin) *in vitro* and *in vivo* had amebacidal activity against *Entamoeba histolytica*. Subsequent clinical studies have indicated that this antibiotic is useful in treating human intestinal amebiasis. Steigman (1953) obtained good results with erythromycin in the treatment of 30 patients with amebiasis. Villarejos (1955) reported good results with the same drug in 98 cases of acute and chronic amebiasis. Nor El-Din (1956) also obtained satisfactory laboratory and clinical response to erythromycin therapy in amebiasis.

Because erythromycin is concentrated in the liver and excreted in the bile in a biologically active form, it was selected for trial in the treatment of amebic liver abscess. Two early cases of amebic liver abscess were treated by Viramuratti and Biseshurarit (1955) with 500 mg of erythromycin every 6 hours for 10 days. Clinical cure resulted in both cases. This is in direct contrast to other broad-spectrum antibiotics, as liver abscess had developed even during treatment with the tetracyclines.

There is no doubt that Elsdon-Dew and his co-workers in the Amebiasis Research Unit in Durban, South Africa, have evaluated more amebacides, including most of the present day antibiotics, in the treatment of acute amebic dysentery than any other group. Due to the large number of cases of acute amebic colitis in the Bantu male of that area, they are able to select patients for therapeutic trials on a strict and uniform basis. The details of the procedure followed for the evaluation of therapeutic trials have been described in a number of reports coming from the Amebiasis Research Unit.

In all of their discussions and evaluations of the antibiotics they indicate that the curative action in amebiasis is mainly through the effect on the associated bacterial flora, although certain antibiotics might have a slight amebacidal action. Fumagillin has been stated to be directly effective against the amebae, with little or no effect on the bacterial flora, yet this antibiotic has been found ineffective in controling acute amebic dysentery.




Graphic 1 — Effect of tetracycline on growth of *Entamoeba histolytica* in modified Shaffer-Frye medium

In a report on the use of antibiotics in acute amebic dysentery, Elsdon-Dew (1954) states that the antibacterial approach has a very definite place in the treatment of acute ulcerative amebic dysentery. It is well known that there are many different bacteria which can support *E. histolytica in vitro*. To be effective against *E. histolytica in vivo* it is apparent that the widest possible antibacterial attack must be used.

There are many who do not agree entirely with the belief that the action of the broad-spectrum antibiotics is entirely on the bacterial flora. The difficulty is to prove that there is direct action on the amebae. Balamuth (1952) and others have pointed up the difficulties in *in vitro* testing for amebacidal activity of any drug since we do not have pure cultures of *E. histolytica*. The use of monobacterial cultures, even though of value, are not definite proof of the direct action of drugs on the parasitic amebae.

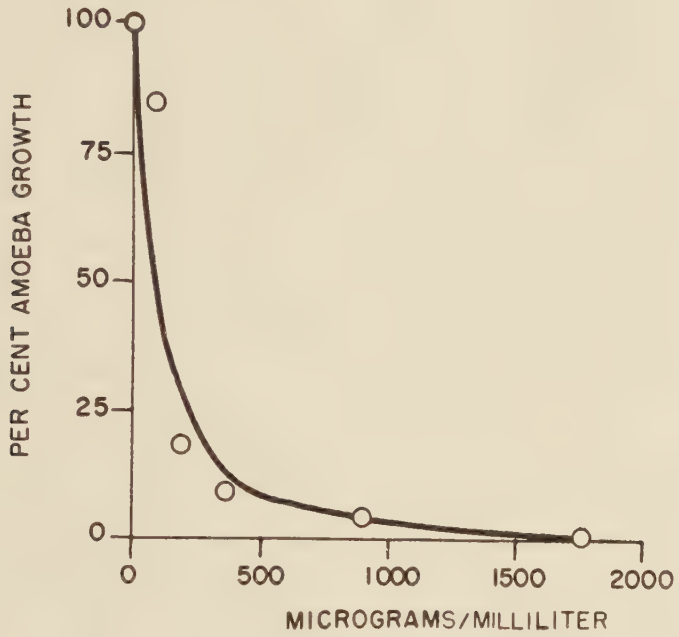
TABLE 3 — *Modified Shaffer-Frye medium*

| | Grams per liter |
|---|---|
| Trypticase (BBL) . . . . . | 20 |
| Glucose . . . . . | 10 |
| Sodium chloride . . . . . | 2.5 |
| $K_2HPO_4 \cdot 3\,H_2O$ . . . . . | 2.0 |
| Thiomalic acid . . . . . | 1.5 |
| Sodium hydroxide to pH 7.0 | $\pm$0.1 |

Space and time do not permit a review of *in vitro* studies to determine the action of the antibiotics on ameba cultures. The bacteria and the constituents of the medium have introduced numerous variables. During the past few years an attempt has been made in our laboratory to develop a technique for growing *E. histolytica* in pure culture. To date this has not been accomplished.

Reeves, Meleney and Frye (1957) reported the development of a modified Shaffer-Frye technique for growing strains of *Entamoeba histolytica* with a penicillin-inhibited monobacterial associate streptobacillus. The constituents of the culture medium are shown in Table 3. The medium base consists of an enzymatic casein digest, glucose, thiomalate and salts dissolved in demineralized water. The original Shaffer-Frye technique was similar but required preconditioning of the medium by growth of the streptobacillus for a period of 24 hours.

The MS-F medium does not require pre-conditioning. The use of a penicillin-inhibited washed suspension of bacterial cells in the ameba cultures allows the maintenance of relatively constant conditions in experiments extending over several days or weeks. Complete inhibition with erythromycin (ilotycin) required over 1,500 μg per milliliter, as shown in Graphic 2.

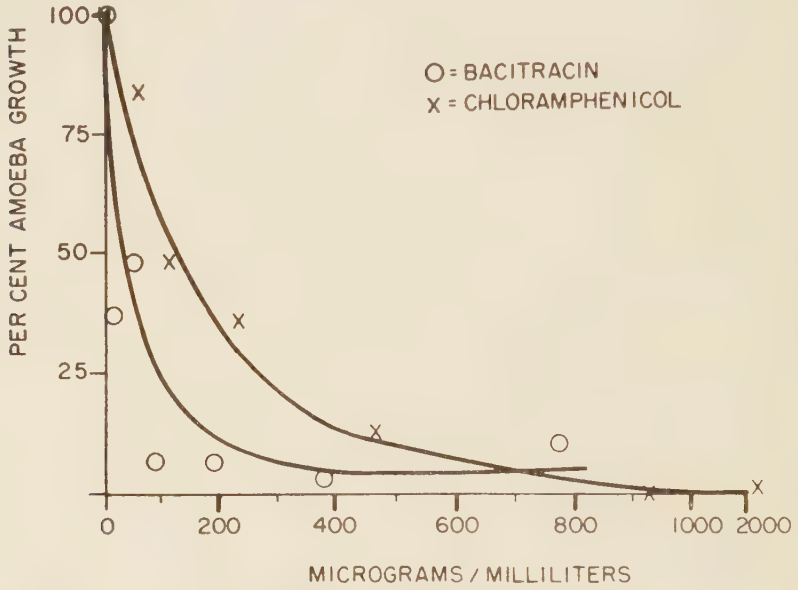



Graphic 2 — Effect of ilotycin (glucoheptonate) on growth of *Entamoeba histolytica* in modified Shaffer-Frye medium

The best results were obtained with tetracycline. There was 50 % inhibition with 10 μg per milliliter and complete inhibition of growth with 40 μg. This broad-spectrum antibiotic, giving the best clinical result, is also more active *in vitro* in the MS-F medium.

These results are by no means conclusive since there is still the penicillin-inhibited non-multiplying bacterial cell in the medium. The results are suggestive and additional studies are being made with different strains of *E. histolytica* in the MS-F medium.

The use of antibiotics and their efficiency in the treatment of amebiasis have been demonstrated. There is still a significant relapse




Graphic 3 — Effect of chloramphenicol and bacitracin on the growth of *Entamoeba histolytica* in modified Shaffer-Frye medium

rate with the best antibiotic available, when used alone. This and the occurrence of amebic hepatitis make it necessary to combine the antibiotics with a systemic amebacide such as emetine or chloroquine.

## BIBLIOGRAPHY

1. Hargreaves, W. H. — «Chronic amebic dysentery. A new approach to treatment», *Lancet*, **2**: 68, 1945.
2. Shaffer, J. G., and Frye, W. W. — «Studies on the growth requirements of *Endamoeba histolytica*», *Amer. Jour. Hyg.*, **47**: 214, 1948.
3. Armstrong, T. G., Wilmot, A. J., and Elsdon-Dew, R. — «The treatment of amebic dysentery in the Bantu African», *Tr. Roy. Soc. Trop. Med. and Hyg.*, **42**: 597, 1949.
4. McVay Jr., L. V., Laird, R. L., and Sprunt, D. H. — «A preliminary report of the successful treatment of amebiasis with aureomycin», *Science*, **109**: 509, 1949.

5. Most, H., and van Assendelft, F. — «Laboratory and clinical observations on the effect of terramycin in the treatment of amebiasis», *Ann. N. Y. Acad. Sc.*, **53**: 427, 1950.
6. Armstrong, T. G., Wilmot, A. J., and Elsdon-Dew, R. — «Aureomycin and amebic dysentery», *Lancet*, **2**: 10, 1950.
7. Tobie, J. E., Most, H., Rearden, L., and Bozicevich, J. — «Laboratory results on the efficacy of terramycin, aureomycin and bacitracin in the treatment of asymptomatic amebiasis», *Amer. Jour. Trop. Med.*, **31**: 414, 1951.
8. Killough, J. H., and Magill, G. B. — «Terramycin in epidemic typhus, amebic dysentery and typhoid», *J. A. M. A.*, **147**: 1737, 1951.
9. Frye, W. W., Brooke, M. M., and Weinstein, P. — «Antibiotics in the treatment of acute amebic dysentery», *Ann. N. Y. Acad. Sc.*, **55**: 1104, 1952.
10. Martin, G. A., Garfinkel, B. T., Brooke, M. M., Weinstein, P. P., and Frye, W. W. — «Comparative efficacy of amebacides and antibiotics in acute amebic dysentery. Used alone and in combination in five hundred and thirty-eight cases», *J. A. M. A.*, **151**: 1055, 1953.
11. Wilmot, A. J. — «A comparison of puromycin and tetracycline and its derivations in amebiasis», *Antibiotics Annual 1955-56*, p. 319.
12. McHardy, G., Mechtold, J. E., Welch, G. E., and Browne, D. C. — «Fumagillin as an amebacide. Preliminary Report», *Southern Med. Jour.*, **46**: 428, 1953.
13. Balamuth, W. — «Comparative action of selected amebacidal agents and antibiotics against several species of human intestinal amebae», *Amer. Jour. Trop. Med. and Hyg.*, **2**: 191, 1953.
14. Sappenfield, R. W., Carter, F. R. N., Culbertson, C., Brooke, M. M., Payne, F. M., and Frye, W. W. — «Therapeutic aspects of a waterborne outbreak of amebiasis in South Bend, Indiana», *J. A. M. A.*, **159**: 1009, 1955.
15. Elsdon-Dew, R., Wilmot, A. J., and Armstrong, T. G. — «Fumagillin in Amebiasis», *Lancet*, **2**: 1180, 1953.
16. McCowen, M. C., Callender; M. E., Lawles Jr.; J. F., and Brandt, M. C. — «The effects of erythromycin (Ilotycin Lilly) against certain parasitic organisms», *Amer. Jour. Trop. Med. and Hyg.*; **2**: 212, 1953.
17. Steigmann, F., Shales, W. H., Mintzer, S., and Jacobson, B. — «The effect of erythromycin in amebiasis», *Jour. Lab. and Clin. Med.*, **42**: 955, 1953.
18. Villarejos, V. M. — «Treatment of amebiasis with erythromycin», *Amer. Jour. Trop. Med.*; **4**: 699, 1955.
19. Nor El-Din, G. — «Erythromycin in the treatment of amebiasis», *Amer. Jour. Trop. Med. and Hyg.*, **3**: 72, 1956.
20. Viramuratti, V., and Biseshurarit, P. — «Clinical trial of erythromycin in amebic liver abscess», *Amer. Jour. Gastroenterol.*, **23**: 127, 1955.
21. Elsdon-Dew, R. — «Antibiotics in acute ulcerative amebic colitis», *Antibiotics Annual 1954-55*, p. 869.
22. Reeves, R. E., Meleney, H. E., and Frye, W. W. — «A modified Shaffer-Frye technique for the cultivation of *Entamoeba histolytica* and some observations on its carbohydrate requirements», *Amer. Jour. Hyg.*, **66**: 56, 1957.

## GENERAL DISCUSSION

Dr. Moisés Mitrani (Cuba) — As representative of Cuba, my mother language is Spanish, but in honor of the distinguished members of this Congress, which will understand better my poor English than my florid Spanish. I will say a few words to express my satisfaction to be in this beautiful Lisbon with her generous people and together with the magnificent representation of the best Doctors on Tropical Medicine in all the world. Certainly, we are proud of the well done work of the Institute of Tropical Medicine of the University of Havana, headed by Profs. Kouri and Basnuevo, my dear friends, expression of what was the very interesting paper presented by Prof. Basnuevo this morning.

In our City Hospital of Havana, in which I am the Director of the Central Laboratory, where we have about 30,000 out patients every year, on whom we make routinely 3 feces tests, we have about 20 % of ameba positives, half of them dysenteric ameba.

In front of this, we have only in the last 10 years reviewed 9 cases of hepatic abscess, all of them operated and treated with antibiotics for microbial infection and chloroquine or emetine, either one or both at the same time for specific treatment for amebiasis. The last one was 3 months ago, which I reported personally to Prof. Basnuevo.

The diagnosis was very difficult and was made after surgical operation. This confirms the few cases recorded by Dr. Basnuevo, of only 72 cases of hepatic ameba abscess in the last 20 years.

In my opinion is that due to the well organized antiparasitic campaign made by the Health Department, in charge of a High Commissioner, Dr. Calvo Fonseca, a real hygienist, with the help of small laboratories in every one of the 125 local Health Departments distributed in Cuba and the care of the different clinics and hospitals we have in the country, where the routine test of feces is a rule. So, we

are able to make early diagnosis in amebiasis, sometimes asymptomatic, and quick treatments, before the complications come. Treatment almost is based in carbarsine, deodoquine, chloroquine and bismuth compounds. Rarely the oxitetracycline is used, due to its high cost and the fine results of the products.

We are in complete agreement with the treatment recommended by Dr. Basnuevo in his paper, and we expect to have the same results with the antibiotics, presented by Prof. Frye in his paper, and in his way we tretated the last case, with complete recovery in 12 days.

I congratulate again Dr. Basnuevo, my dear friend, and I want to express the deep gratitude of my country to the Organizing Committee, for confering to a distinguished Cuban parasitologist, Dr. Basnuevo, the honor and responsibility to be a Relator or Rapporteur in this subject.

Dr. François Blanc (France) — La seule difficulté du diagnostic de l'amibiase hépatique n'est pas de la reconnaître mais d'affirmer le stade lésionnel: amibiase pré-suppurative: semis d'îlots nécrotiques primaires ou abcès collecté.

Hors les rares cas d'abcès collecté, évident physiquement ou radiologiquement, il est impossible à l'examen clinique le mieux conduit de déterminer le stade lésionnel.

La ponction exploratrice peut ne pas rencontrer l'abcès parfaitement existant et quand elle le rencontre elle conduit à l'exploration à chaud.

L'attitude clinique et thérapeutique valable est à notre sens la suivante:

Abcès cliniquement ou radiologiquement évident en imminence de migration dangereuse ou de rupture: intervention et traitement amoebicide;

Abcès cliniquement évident non en imminence de migration et de rupture: traitement amoebicide, puis intervention à froid;

Amibiase hépatique dont il est impossible de reconnaître le stade lésionnel: traitement spécifique et contrôle quotidien de la mesure de la vitesse sédimentaire des hématies:

*a*) Correction de la VSH: il s'agit d'une amibiase hépatique pré-suppurative parfaitement guérie par le traitement;

*b*) Non correction de la VSH (tous les signes et tous les symptomes ont cependant disparu): il existe un abcès du foie central collecté que la ponction, réellement exploratrice cette fois, découvrira: intervention à froid.

Nous sommes resté fidèles à l'émétine, seul amoebicide diffusible de haute valeur. Employée par voie sous-cutanée à posologie raisonnable, elle est sans danger (la durée de son accumulation doit être évidemment connue).

Les antibiotiques n'ont aucune action directe sur l'amibe. Ils améliorent les colites amibiennes parce qu'ils tuent les flores associées dont le role est prédominant. Ils ne font rien sur l'amibiase hépatique où l'amibe est seule en cause.

On pourrait concevoir la recherche d'un antibiotique efficace à l'endroit de l'amibe à partir de champignons faisant dans la nature leur pature des amibes libres, si tant est que de tels champignons existent.

Dr. Jean Schneider (France) — Le sentiment général est que les antibiotiques n'ont pas d'action anti-amibienne directe et que leur succès est due à leur action sur la flore amibienne associé, dont le rôle est considérable dans la pathogénie de la maladie.

On comprend que plus le spectre d'action de l'antibiotique est large, plus grande sera l'action de la thérapeutique sur la résistance de l'amibe, alors privée de son support microbien, et c'est à notre avis l'explication du succès remarquable des traitements par les tétracyclines.

Mais il est une rançon à l'emploi de ces antibiotiques; nous connaissons l'existence, rare heureusement, de cas où à la suite de traitement par une tétracycline la destruction massive de la flore intestinale a permis la selection soit de monilias, soit de staphylocoques résistants à cet antibiotique, germes qui sont responsables parfois de syndromes cholériformes redoutables.

La crainte de tels accidents, pour rares qu'ils soient, nous incite à la prudence et nous refusons de risquer ainsi la vie de malades souvent peu atteints avec de tels traitements. C'est pourquoi nous pensons qu'il est plus sage d'employer des antibiotiques tels que l'érythromycine ou la spiramycine actifs sur la flore microbienne gram-positive (y compris les anaérobies dont on connait le rôle pathogénique important) et de les associer à un médicament véritablement amoebicide (émétine, arsenicaux, etc.).

Ainsi, sans faire courir de risques au malade, nous obtenons par cette association des résultats aussi rapides et aussi durables que ceux qui suivent un traitement par la tétracycline.

Dr. J. Schneider (France) — À propos des formes diarrhéiques prolongées de l'amibiase intestinale attire l'attention sur la possibilité existant d'observer un cancer intestinal ou une recto-colite hémorragique chez un ancien amibien. Aussi, il importe de ne pas s'arrêter trop longtemps à la notion d'amibiase et il faut penser à d'autres causes des formes diarrhéiques de longue durée. S. a eu à diverses reprises l'occasion de faire de tels diagnostics chez des malades traités sans succès trop longtemps par des médications anti-ambiennes et chez qui une intervention chirurgicale faite en temps utile aurait permis qui une intervention chirurgicale faite en temps utile aurait permis souvent de guérir le malade.

Dr. José Waks (Argentina) — Since 1956 we have used 11925 C to investigate it experimentally and clinically in amoebiasis.

Our experience amounts to 248 patients with confirmed amoebiasis. All the patients were studied according to the following plan:

Clinically
- Physical examination
  - General condition
  - Weight
- Analysis every 3 days
  - Urine
    - Albumine
    - Sediment
    - Addis sediment count
  - Blood
    - Hemogram
    - Prothrombine time
    - Urea
    - Glycemia
    - Hanger reaction
- Study of the alimentary regime

Coprologically, dayly controls for 20 days. Then every 15 days during 1 year
- Function study of digestive capacity
- Bacteriological study
- Parasitological study
  - Flotation as Faust
  - Staining { Donaldson / D'Antoni }
  - Hematoxiline (Man-Dobell)
  - Culture and passage by inoculation into the sigmoid of the kitten

*Dosage:* The adult patients received:
1st to 5th day: 100 mg (2 tablets) after lunch.
6th to 15th day: 100 mg.

Dr. A. H. Mousa (Egypt) — I like to stress the difference between the ameboma of caecum and that of rectum: in the former it is boggy and friable while in the latter it is fibrous. I also noticed that cases of enterica in association of amoebic infection make such cases vulnerable to intestinal complications. The association of anti-amoebic treatment with chloramphenicol minimized these complications.

I had also the experience of putting cases going to heavily endemic areas to be put on long chemoprophylaxis with encouraging results in minimizing the manifestations of amoebiasis among them.

In my experience in Egypt in treatment of liver abscess I found penicillin is the best antibiotic to use.

In cases of hepatic bilharziasis, when they get hepatic amoebiasis as well the clinical and laboratory findings are modified and emetine has to be avoided as a therapeutic test as it is antibilharzial as well as anti-amoebic, chloroquine is hence preferable.

Dr. Leon Jacobs (U.S.A.) — I should like to point out, for the record, some of the early studies on the effects of antibiotics on *E. histolytica* that were performed at the National Institute of Health in Bethesda, Md., U.S.A.

As early as 1941, I was able to show that penicillin is ineffective against *E. histolytica in vitro*. This was recorded in a note by Dr. C. W. Rees, published in *Tropical Medicine News* in 1943 or 1944. Similarly, streptomycin was later found ineffective against the amoeba, and these two antibiotics were useful in studies in which the bacterial flora of cultures could be altered by the judicious use of

these drugs, depending on the susceptibility of the various bacteria involved (Jacobs, 1947, *Am. J. Hyg.*; Jacobs, 1950, *J. Parasitol.*).

One of the most interesting demonstrations of the effects of antibiotics on *E. histolytica* was provided by the work of Phillips, who used amoeba — *Trypanosoma cruzi* cultures, established by micro-isolation, to test the effects of drugs *in vitro*. He was able to show, by this method, a direct amoebicidal effect of tetracycline antibiotics on *E. histolytica*.

Dr. G. Piganiol (France) — Malgré la valeur des traitements médicaux dont on dispose contre l'amibiase, la thérapeutique chirurgicale garde un plan devant certaines complications de la maladie. Cette place que d'aucuns croient pouvoir diminuer, quelle est elle exactement? La chirurgie est indiquée quand deux conditions sont remplies: possibilité d'une intervention efficace, échec du traitement médical.

Dans les complications chroniques de l'amibiase il est toujours possible de prolonger le traitement médical jusqu'à ce qu'il ait vraiment fait la preuve de son efficacité ou de son inefficacité. C'est le cas des complications directement amibiennes, comme les amoebomes (qui guérissent presque toujours médicalement), ou para-amibiennes, comme les ulcères gastroduodenaux des amibiens.

Dans les complications subaiguës, comme les abcès du foie, la place de la chirurgie est plus délicate à situer justement. L'efficacité du traitement médical est certaine. Des abcès guérissent par l'association traitement médical-ponction. Mais dans bien des cas il rend l'abcès muet sans le faire disparaître. L'indication opératoire *ubi pus, ibi evacua* reste essentielle, particulièrement difficile chez des abcès que la thérapeutique médicale a su refroidir sans guérir et qui continuent à augmenter à bas bruit.

Dans les complications aiguës du type péritonite par perforation on se peut attendre l'échec du traitement médical.

On sait les difficultés de l'acte opératoire. Aux perforations lenticulaires simples s'opposent ces cas fréquents où un intestin nécrotique se déchire comme un «chiffon usé» sous la main du chirurgien.

Il me semble cependant difficile de refuser le secours de la chirurgie à une péritonite par perforation du gros intestin chez tout malade capable de suporter une laparotomie. Cet act opératoire sera

complété par un traitement amoebicide, antibiotique et d'une rééquilibration générale. Pour les *morituri,* chez lesquels nous renoncerons à tout acte chirurgical, ou après l'intervention chez les malades graves, il faut penser à l'efficacité du traitement neuroplegique et de l'hibernation artificielle qui permet à certains malades de passer le cap d'une complication trop grave. J'ai plaisir à conclure comme un des rapporteurs: abstenons-nous autant que possible de la chirurgie sauf pour les malades qui en ont vraiment besoin pour guérir.

**FREE COMMUNICATIONS OFFERED TO THE SUBSECTION 2 — AMOEBIASIS, WHICH COULD NOT BE PRESENTED AND DISCUSSED DUE TO THE SHORTAGE OF TIME ALLOWED FOR THE PROGRAMME**

# INGESTION OF BACTERIA BY *ENTAMOEBA HISTOLYTICA*

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For many years it has been realized that bacteria can supply factors or conditions essential for the growth and multiplication of *Entamoeba histolytica* in culture. Experimental evidence that this is true also in the animal host was shown by the studies of Phillips *et al.* (1955) on *E. histolytica* infections in germ free guinea pigs. In many aspects of the life of this ameba bacteria appear to have a role. In the earliest *in vitro* studies evidence was obtained that bacteria provide factors aiding grouth and multiplication. The role of bacteria has been under investigation for many years and in their recent studies Rees *et al.* (1953), Phillips and Bartgis (1954) and Baernstein, Rees and Reardon (1954) suggest that a symbiotic relationship exists. Bacterial associates may also influence encystment, as shown by Chang (1942) and others. Excystation is promoted by the presence of suitable bacteria, as shown by Meleney (1940), Snyder *et al.* (1941) and others. Evidence provided by the studies of Luttermoser *et al.* (1952) has shown notable influence of bacterial associates on the infectivity of the organisms and finally even the virulence and the pathogenesis of tissue invasion are markedly influenced by bacteria, as shown by Phillips *et al.* (1955).

In many of these studies, as summarized in the recent reports of Rees *et al.* (1953) and Baernstein *et al.* (1954), it has been found

that certain species of bacteria are superior to others in providing for the varied aspects of life of *E. histolytica*. The specific mechanism involved remains obscure. For the most part the problem has been approached from the viewpoint that the relationship is essentially a beneficial environmental association. Undoubtedly the associative viewpoint was furthered by the old concept that *E. histolytica* does not normally feed on bacteria. As a result, the search for evidence on the role of bacteria has been concentrated on changes and conditions arising in the constituents of the medium. It is not surprising that observations on bacterial ingestion have been in abeyance. The circumstances leading to recent renewal of the study of the bacteria ingesting activities have been well summarized by Hoare (1952) in his reports presenting a forceful case for the existence of a commensal, bacteria ingesting phase of *E. histolytica*. Similarly, Shaffer (1958) has brought the problem into focus with his study on the ingestion activities of *E. histolytica* grown in association with a single species of bacteria. It may well be that within this realm will be found the specific mechanism in the role of bacteria in the life of *E. histolytica*.

In this preliminary study we have attempted to ascertain the feasibility of applying a number of techniques to the study of the bacteria ingesting activity of *E. histolytica*. It has become apparent to us that ingested bacteria are often inconspicuous and easily overlooked even though present in considerable number and that the commonly used preparations and methods of observation are inadequate. The difficulties are due, on the one hand, to the peculiarly refractile character of the ectoplasmic layer of this ameba and, on the other, to the inconspicuous character of certain bacteria. The ectoplasmic layer of the ameba usually hides even the nucleus of the living ameba. Even with the phase microscope ingested bacteria are made out with difficulty in the living ameba.

A series of tests with a variety of vital dyes proved helpful. The most useful vital stain was found to be a 0.1 % solution of crystal violet in 0.001 NHCl. Certain bacteria were discerned in the amebae before cytoplasmic staining and death of the amebae ensued.

Helpful data were obtained from these temporary preparations, but we felt the obvious need for adequate permanent preparations. In our experience hematoxylin and other stains ordinarily used in routine study of *E. histolytica* are inadequate for critical determination

of the bacterial content of the ameba cell. Gram stain proved to be of limited value, but proved that our problem was largely concerned with revealing gram-negative bacteria. For staining, gram-negative bacteria in cells, Lillie (1954) recommended Giemsa and related stains. It became apparent from a series of trials that the major requirement is the prevention of distortion and objectional granulation in the cytoplasm of the amebae. For this reason commonly used fixing methods proved inadequate. The solution was finally reached in tests which showed that Lugol's and similar iodine solutions accomplished instant killing and apparently a mild fixation which favored the production of well stained preparations.

The following procedure was devised for routine use. A drop of culture or other material containing active amebae is placed on a clean slide. A small amount of Lugol's iodine solution is added and the materials mixed and spread in a film and dried in an incubator. When dry, the preparation is rinsed for a minute in a solution of equal parts of alcohol and acetone. The film is then stained for 3 to 6 minutes in 1 to 10 Giemsa in pH 6.8 buffered distilled water. The stained film is rinsed in buffered water, dried and examined under oil. The ameba cytoplasm stains a pale blue and bacteria violet.

In this study we have made observations on four strains of *E. histolytica*. Strains G and M were isolated from cases of amebic dysentery, strain SA from a case of diarrhoea and strain SM from a carrier. Strain SM is a small race and the others are large. These strains have been maintained in culture for periods ranging from a few months to 10 years in the media of Nelson (1947) and Nelson and Jones (1955). Each strain is accompanied by a mixed bacterial flora presumably derived from the original inoculum.

This report deals primarily with the results obtained from a series of preparations made from cultures at the time of active growth on the third day. Concomitant studies have been made on the bacterial associates involving isolation of pure cultures and staining for observation of morphology. In certain instances species identification has been carried to completion. Strain G cultures, for example, contain a heavily encapsulated *Aerobacter*, *Escherichia coli*, a *Pseudomonas*, a filamentous granular gram-positive rod, a streptococcus and possibly other less conspicuous species. The bacterial flora accompanying the other strains appears to be composed of an essentially similar array.

After careful study of a number of series of preparations from our cultures it became apparent that, with this small number of morphological types of bacteria present, a basis of estimating bacteria ingesting activity is available. Studies on our four strains have revealed discernable differences which suggest a number of tentative conclusions.

Strain G and M feed almost entirely on the encapsulated *Aerobacter*. Other species of bacteria occur so rarely and in such small numbers that their ingestion appears to be incidental. Strain SA feeds almost entirely on a slender, granular gram-positive bacillus. Strain SM, the small race *E. histolytica*, ingests few bacteria and limits its ingestion almost entirely to a small gram-negative rod. Within our series there thus appear three ingestion types. These differences apparently remain constant. The feasibility of using this procedure for extended study has been established. Our series is too small to provide more than tentative suggestions, but certain questions can be raised as a result of the findings.

The differences in staining noted by us may provide an explanation for the failures encountered in attemps to establish certain strains of *E. histolytica* in culture with bacteria which are known to be adequate for the maintenance of other strains (Nakamura. 1952; Faust *et al.*, 1950). Our observations also suggest the possibility that a study of the bacteria ingesting activity of ameba strains in culture with a mixed flora may provide a basis for selection of appropriate species of bacteria for the establishment of monobacterial cultures.

Many studies have directed attention to the adverse effect of too many bacteria in the medium. The reasons are as yet obscure, but it is apparent from our observations that if the specific mechanism of growth lies in the ingested bacteria, then a small number should suffice. A single ameba will occasionally contain many bacteria, but in general the number is less than 20.

We know nothing of the fate of ingested bacteria. It is possible for example that an encapsulated *Aerobacter* is divested of its capsule and discarded. We do not know how long ingested bacteria are retained or if they are discharged in a viable state. This latter possibility is of importance in relation to the problems of infectivity and tissue invasion.

It may also have a bearing on the establishment of cultures by trophozoite inoculation. These are but a few of the questions inherent in the bacteria ingesting activity of *E. histolytica.*

The preliminary observations made in this study suggest the feasibility of this approach to the study of the bacteria ingesting activity. It is apparent that *E. histolytica* often ingests bacteria in large numbers and that these can be viewed by applying appropriate procedures. Our findings suggest that feeding may be selective in some measure and that strains may differ in their selection of bacteria from a mixed flora. It is suggested that the mechanism of the role of bacteria in the life of *E. histolytica* may reside specifically with the ingested bacterium.

## BIBLIOGRAPHY


CHANG, S. L. — *Am. J. Trop. Med.*, **22**: 471-485, 1942.

BAERNSTEIN, HARRY D.; REES, CHARLES W., and REARDON, LUCY V. — *Am. J. Trop. Med.* **3**: 839-848, 1954.

FAUST, E. C., SADUN, E. H., EVERRITT, M. C., BRADIN, J. L., and LEWIS, R. A. — *Jour. Parasit.*, **36**: 336-342, 1950.

HOARE, C. A. — *Parasitology*, **42**: 43-47, 1952.

HOARE, C. A. — *Exp. Parasitology*, **1**: 411-427, 1952.

LILLIE, R. D. — *Histopathologic Technique and Practical Histochemistry*, The Blakiston Company, New York, 1954.

LUTTERMOSER, GEORGE W., and PHILLIPS, BRUCE P. — *Am. J. Trop. Med. and Hyg.*, **1**: 731, 1952.

MELENEY, H. E., FRYE, W. W., LEATHERS, W. S., and SNYDER, T. L. — *Proc. Third Intemat. Cong. Microbiology*, New York, Sept. 1939, pp. 410-411, 1940.

NAKAMURA, M. J. — *Parasitology*, **38**: 83, 1952.

NELSON, E. C. — *Am. J. Trop. Med.*, **27**: 545, 1947.

NELSON, E. C., and JONES, M. M. — *Am. J. Trop. Med. and Hyg.*, **4**: 822, 1955.

PHILLIPS, B. P., and BARTGIS, I. L. — *Am. J. Trop. Med. and Hyg.*, **3**: 621, 1954.

PHILLIPS, B. P., WOLFE, PATRICIA, REES, C. W., GORDON, H. A., WRIGHT, W. H., and REYNIERS, J. A. — *Am. J. Trop. Med. and Hyg.*, **4**: 675, 1955.

REES, CHARLES W., BAERNSTEIN, HARRY D., REARDON, LUCY V., and PHILLIPS, LAURA — *Am. J. Trop. Med.*, **2**: 1002, 1953.

SHAFFER, J. G., SCHULER, R. W., and KEY, I. D. — *Am. J. Trop. Med. and Hyg.*, **7**: 302, 1958.

SNYDER, T. L., and MELENEY, H. E. — *Am. Jour. Trop. Med.*, **21**: 63-69, 1941.

# THE EFFECT OF SIGNEMYCIN [1] AGAINST [2] EXPERIMENTAL AMEBIASIS IN RATS

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## INTRODUCTION

*In vivo* studies and clinical trials with antibiotics primarily effective against bacterial species, which also demonstrated anti-amebic activity, indicated that their effectiveness was due to a modification of the bacterial flora of the bowel, resulting in a change in the environment of the ameba (1, 2).

Studies with tetracycline [3] and oleandomycin [3] against *Entamoeba histolytica* (3, 4) *in vivo*, and subsequent clinical trials with tetracycline (5, 6) have demonstrated that these antibacterial antibiotics possess significant anti-amebic activity, probably brought about by the inhibition of the enteric bacteria primarily important in the development of intestinal amebiasis. The relative ineffectiveness of tetracycline and oleandomycin against *E. histolytica in vitro* has been found in this and other laboratories (3, 4).

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[1] The trade name of Chas. Pfizer and Co., Inc., for the combination of 67 % tetracycline and 33 % oleandomycin is Signemycin.

[2] This paper, under the title «The effect of tetracycline-oleandomycin (Signemycin) against experimental amebiasis in rats», has been published in *Antibiotics & Chemotherapy*, **10** (5): 294-300, 1959.

[3] The trade name of Chas. Pfizer and Co., Inc., for the antibiotic tetracycline is Tetracyn; for the antibiotic oleandomycin the trade name is Matromycin.

The combined effect of tetracycline and oleandomycin against laboratory induced amebiasis in weanling rats will be presented in this report.

## MATERIALS AND METHODS

Tetracycline base and oleandomycin base were used throughout this study on an activity basis. Micronized suspensions of the antibiotics were prepared in distilled water. The comparative activity of the mixture of tetracycline and oleandomycin, its component parts, and the same total amounts of the individual antibiotics in the combination were measured against the experimental infection. To illustrate: the activity resulting from doses of 300, 200, 100, 50, 25, 12.5. and 6.25 mg/kg of the combination was compared to the activity of tetracycline at 201, 134, 67, and 33.5 mg/kg. Oleandomycin was evaluated at 99, 66, 33. and 16.5 mg/kg. In addition, infected animals were treated with amounts of the component antibiotics comparable to the total dose of the mixture.

The rats used in this study were 19-day old female albinos weighing 35 g to 40 g at the time of inoculation. The 200 F strain of *E. histolytica* (4) was used in all experiments. The culture was maintained on Locke's egg slant-Locke's overlay medium in the presence of an undetermined flora. The inoculum was prepared from pooled 48 hour cultures containing approximately 300,000 trophozoites per 1.0 ml. The rats were inoculated intracecally with 0.3 ml of the pooled culture after laparotomy.

The antibiotic preparations were administered once daily for 5 consecutive days, beginning approximately 24 hours after inoculation. The efficacy of therapy was evaluated approximately 48 hours after the fifth treatment, which was the eighth day after inoculation. Autopsies were performed on all rats for macroscopic and microscopic comparisons between treated and non-treated groups. These examinations afforded a means for determining the proportion of percent cleared, the average degree of infection (A.D.I.) and percent efficiency. The A.D.I. is based on the following scheme:

1. Few amebae.
2. Many amebae, no inflammation of the cecum.

3. Amebae present, inflammation of the cecum.
4. Amebae present, inflammation and/or ulceration of the cecum, mucous present.

By comparing the A.D.I. of treated groups with that of the infected controls, it is possible to find a numerical value designated «percent efficiency» for each experimental group. The percent efficiency is determined by dividing the A.D.I. of the infected controls into the difference between the A.D.I. of the infected controls and any given treated group, and then multiplying the value obtained by 100 to determine percent value. This serves as an expression of the ability of a particular dosage level to decrease infection.

Eleven experiments were conducted using 846 rats. In each experiment infected controls were used for comparative purposes, and in most experiments where the combined antibiotics were studied, the component antibiotics were also included as part of that experiment.

## RESULTS AND DISCUSSION

The therapeutic activity of tetracycline and/or oleandomycin was based on the combined observations of the authors at the time of sacrifice and autopsy. Gross pathological appearance of the cecum of each rat was noted, scrapings from the cecal mucosa were made for microscopic examination, and after verification of the gross appearance and microscopic examination of the cecal scrapings, the degree of infection decided upon was recorded.

The results of the study are presented in summary form in Table 1. The anti-amebic activity of each regimen considered is expressed on the basis of clearance of infection and suppression of infection. The comparative activities of component parts and component levels equal to the total of the combined levels are clearly expressed because in each instance there is a marked difference in activity between the antibiotic mixture and the sum of the parts, and the individual components at equal levels.

Tables 2 through 5 have been included to afford the opportunity of studying the complete laboratory data obtained from these experiments, more particularly the comparative results obtained from each

TABLE 1 — *A comparative study of the effect of tetracycline and oleandomycin alone and in combination against experimental amebiasis in rats*

| Compound(s) | Levels mg/kg | Number of trials | Number of rats inoculated | Number of rats cleared | Percent cleared | A.D.I. | | Percent efficiency |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Treated | Infected controls | |
| Tetracycline + oleandomycin | 300 | 4 | 47 | 42 | 89 | 0.2 | 3.0 | 93 |
| Tetracycline | 201 | 3 | 36 | 7 | 19 | 2.3 | 3.3 | 30 |
| Oleandomycin | 99 | 3 | 37 | 9 | 24 | 2.0 | 3.3 | 39 |
| Tetracycline + oleandomycin | 200 | 4 | 46 | 39 | 85 | 0.3 | 3.0 | 90 |
| Tetracycline | 134 | 4 | 51 | 6 | 12 | 2.5 | 3.3 | 24 |
| Oleandomycin | 66 | 4 | 50 | 6 | 12 | 2.2 | 3.3 | 33 |
| Tetracycline + oleandomycin | 100 | 4 | 49 | 37 | 76 | 0.6 | 3.0 | 80 |
| Tetracycline | 67 | 3 | 38 | 5 | 13 | 2.8 | 3.3 | 15 |
| Oleandomycin | 33 | 3 | 35 | 2 | 5.7 | 2.9 | 3.1 | 6.5 |
| Tetracycline + oleandomycin | 50 | 4 | 50 | 33 | 66 | 0.8 | 3.0 | 73 |
| Tetracycline | 33.5 | 3 | 34 | 1 | 2.9 | 3.2 | 3.3 | 3.0 |
| Oleandomycin | 16.5 | 2 | 26 | 1 | 3.9 | 3.0 | 3.1 | 3.2 |
| Tetracycline + oleandomycin | 25 | 3 | 37 | 22 | 59 | 1.1 | 3.5 | 69 |
| Tetracycline + oleandomycin | 12.5 | 2 | 23 | 10 | 43 | 1.4 | 3.0 | 53 |
| Tetracycline + oleandomycin | 6.2 | 2 | 22 | 1 | 4.5 | 2.6 | 3.0 | 13 |
| Tetracycline | 300 | 3 | 37 | 12 | 32 | 2.3 | 3.2 | 28 |
| | 100 | 3 | 32 | 6 | 19 | 2.7 | 3.2 | 16 |
| Oleandomycin | 300 | 3 | 36 | 21 | 58 | 1.3 | 3.2 | 59 |
| | 200 | 3 | 38 | 16 | 42 | 1.6 | 3.2 | 50 |

TABLE 2 — *Degree of infection for each rat treated with tetracycline-oleandomycin combination (67 %-33 %)*

| | 300 mg/kg | | | | 200 mg/kg | | | | 100 mg/kg | | | | 50 mg/kg | | | | 25 mg/kg | | | 12.5 mg/kg | | 6.25 mg/kg | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Trial number | | | | Trial number | | | | Trial number | | | | Trial number | | | | Trial number | | | Trial number | | Trial number | |
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 1 | 2 | 1 | 2 |
| Individual rats | 0 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 3 | 4 | 3 | 4 | 2 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 3 | 4 | 4 |
| | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 2 | 2 | 2 | 2 | 4 | 2 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 4 |
| | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 3 | 3 | 1 | 3 | 3 | 2 | 3 | 2 | 3 | 3 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 | 0 | 0 | 3 | 2 | 2 | 3 | 2 | 3 | 3 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 3 | 2 | 0 | 3 | 2 | 3 | 3 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 3 | 2 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 2 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 2 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | |
| | 0 | | 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 2 | |
| | 0 | | 0 | 0 | | | 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 2 | |
| | | | | 0 | | | | 0 | | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | | | 0 | | 2 | |
| | | | | | | | | | | | | 0 | | 0 | | | | | | | | | |
| Number of rats | 12 | 10 | 12 | 13 | 10 | 11 | 12 | 13 | 9 | 13 | 13 | 14 | 10 | 14 | 13 | 13 | 13 | 12 | 12 | 13 | 10 | 13 | 9 |
| Experiment number | 1 | 2 | 3 | 5 | 1 | 2 | 3 | 5 | 1 | 2 | 4 | 6 | 1 | 2 | 4 | 6 | 4 | 7 | 8 | 10 | 11 | 10 | 11 |

TABLE 3 — *Degree of infection for each rat treated with tetracycline alone*

| | 300 mg/kg | | | 201 mg/kg | | | 134 mg kg | | | | 100 mg/kg | | | 67 mg/kg | | | 33.5 mg/kg | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Trial number | | | Trial number | | | Trial number | | | | Trial number | | | Trial number | | | Trial number | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Individual rats | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 3 | 3 | 4 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 3 | 4 | 4 | 4 |
| | 4 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 3 | 4 | 4 | 3 |
| | 4 | 4 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 4 | 3 | 3 | 4 | 3 |
| | 3 | 4 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 4 | 3 | 3 | 4 | 3 |
| | 3 | 4 | 2 | 3 | 2 | 3 | 2 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 4 | 3 |
| | 0 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 0 | 3 | 3 | 3 | 4 | 3 | 3 | 4 | 3 |
| | 0 | 3 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 0 | 2 | 3 | 2 | 3 | 2 | 2 | 3 | 3 |
| | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 2 | 2 | 2 | 0 | 0 | 2 | 2 | 3 | 2 | 1 | 3 | 2 |
| | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 1 | 1 | | | 0 | 2 | 3 | 2 | | 3 | 2 |
| | | 0 | 0 | | 0 | 0 | 2 | 0 | 0 | 0 | | | 0 | 0 | 0 | 0 | | 2 | |
| | | | 0 | | 0 | | 2 | 0 | 0 | | | | | 0 | 0 | | | 0 | |
| | | | 0 | | | | | | | | | | | | | | | | |
| Number of rats | 11 | 12 | 14 | 11 | 13 | 12 | 13 | 13 | 13 | 12 | 10 | 10 | 12 | 13 | 13 | 12 | 10 | 13 | 11 |
| Experiment number | 8 | 9 | 10 | 3 | 5 | 7 | 2 | 3 | 5 | 7 | 8 | 9 | 10 | 4 | 6 | 7 | 4 | 6 | 7 |

TABLE 4 — *Degree of infection for each rat treated with oleandomycin alone*

| | 300 mg/kg | | | 200 mg/kg | | | 99 mg/kg | | | 66 mg/kg | | | | 33 mg/kg | | | 16.5 mg/kg | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Trial number | | | Trial number | | | Trial number | | | Trial number | | | | Trial number | | | Trial number | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 1 | 2 |
| Individual rats | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 3 | 3 | 4 | 3 | 3 | 4 | 4 | 4 | 3 | 4 | 3 | 4 | 4 | 3 | 4 | 4 |
| | 4 | 4 | 2 | 3 | 4 | 3 | 3 | 4 | 3 | 3 | 3 | 4 | 3 | 4 | 4 | 3 | 4 | 4 |
| | 0 | 4 | 2 | 3 | 4 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 3 | 4 | 4 |
| | 0 | 2 | 2 | 2 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 2 | 4 | 4 | 3 | 4 | 4 |
| | 0 | 2 | 0 | 1 | 1 | 2 | 2 | 3 | 2 | 3 | 3 | 3 | 2 | 3 | 4 | 3 | 4 | 3 |
| | 0 | 2 | 0 | 0 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 4 | 3 | 3 | 3 |
| | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 3 |
| | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 3 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 1 | 2 | 2 | 2 | 3 | 1 | 1 | 3 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 1 | 1 | 3 | | 1 | 3 |
| | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 1 | 0 | 0 | 1 | | 0 | 3 |
| | | | 0 | | | 0 | | 0 | | | | 0 | | | 0 | | | 3 |
| | | | | | | 0 | | 0 | | | | 0 | | | | | | 2 |
| Number of rats | 11 | 12 | 13 | 12 | 12 | 14 | 12 | 14 | 11 | 12 | 12 | 14 | 12 | 12 | 13 | 10 | 12 | 14 |
| Experiment number | 8 | 9 | 10 | 8 | 9 | 10 | 3 | 5 | 7 | 2 | 3 | 5 | 7 | 4 | 6 | 11 | 4 | 6 |

TABLE 5 — *Degree of infection for each rat used as a control*

| | Experiment number | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| Individual rats | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 |
| | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 |
| | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 |
| | 2 | 3 | 3 | 4 | 3 | 3 | 4 | 4 | 4 | 3 | 2 |
| | 2 | 3 | 3 | 4 | 3 | 3 | 4 | 4 | 4 | 3 | 2 |
| | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | |
| | 2 | 2 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | |
| | 2 | | 2 | 0 | 3 | 2 | 3 | 3 | 2 | 3 | |
| | | | 2 | | 3 | 2 | 3 | 3 | 1 | 2 | |
| | | | 0 | | 3 | 2 | 3 | | 0 | 2 | |
| | | | | | 2 | 0 | | | | 2 | |
| Number of rats | 10 | 9 | 12 | 10 | 13 | 13 | 12 | 11 | 12 | 13 | 7 |

rat in each experiment and for each trial, for all regimens studied. An analysis of any level permits the investigator to check the infected controls used in the same experiment and to observe the activity of other regimens studied under identical conditions. Usually 6 levels were evaluated in each experiment and, wherever possible, the component parts of a particular combination level were evaluated as part of the same experiment.

In all cases where the mixture is compared with the sum of its parts, or to its parts at the same total concentration, a noticeable difference is evident, either by a comparison of percent clearance or percent efficiency. Figures 1 and 2 show graphically activity against experimental amebiasis in rats which is considerably greater than can be accounted for by either tetracycline or oleandomycin *per se*.

In clinical studies Loughlin and Mullin (7, 8) found that the tetracycline-oleandomycin combination was the most effective agent they had used against chronic intestinal amebiasis.

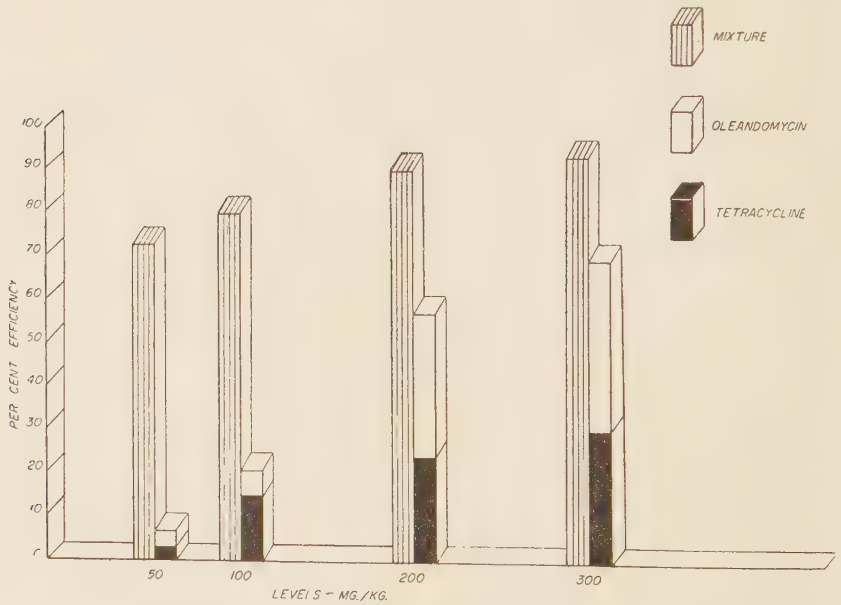


Fig. 1 — Comparison of the mixture of tetracycline and oleandomycin with the sum of its parts

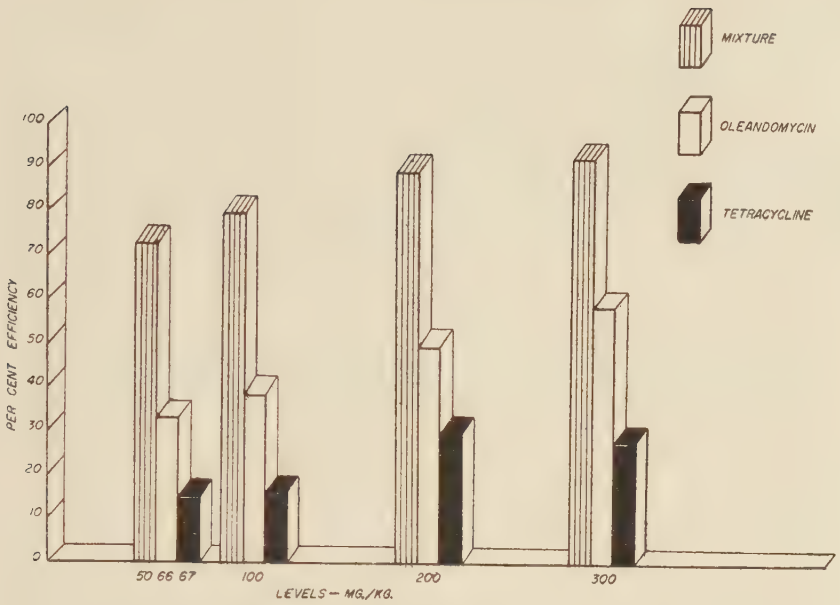


Fig. 2 — Comparison of the mixture of tetracycline and oleandomycin with equal concentrations of its components

## SUMMARY

Tetracycline and oleandomycin, two antibiotics primarily effective against bacterial species, have been evaluated for anti-amebic activity in laboratory infected weanling rats.

A physical mixture of 67 % tetracycline and 33 % oleandomycin (Signemycin) exhibited markedly higher activity at every level studied than each antibiotic when studied at the levels contained in the mixture, or at levels equal to the total of the combination.

## BIBLIOGRAPHY

1. Armstrong, T. G., Wilmot, A. J., and Elsdon-Dew, R. — *The Lancet,* July 2, 14, 1955.
2. Anderson, H., Nelson, T., Hrenoff, A., and Fish, C. — *American Journal of Tropical Medicine and Hygiene,* **3**: 2, 1954.
3. Lynch, J. E., Bamforth, B. J., and Goeckeritz, D. — *Antibiotics and Chemotherapy,* **6**: 5, 1956.
4. Thompson, P., McCarthy, D., Bayles, A., Reinertson, J., and Cook, A. — *Antibiotics and Chemotherapy,* **6**: 5, 1956.

5. Wilmot, A. J. — *Antibiotics Annual 1955-1956,* Medical Encyclopedia, Inc., New York, 319, 1956.
6. Sanchez, F., Sanchez, A., Cham, J., and Granda, E. — *Antibiotic Medicine and Clinical Therapy,* **4**: 8, 1957.
7. Loughlin, E., and Mullin, W. — *Antibiotics Annual 1956-1957,* Medical Encyclopedia, Inc., New York, 63, 1957.
8. Loughlin, E., and Mullin, W. — *Antibiotics Annual 1957-1958,* Medical Encyclopedia, Inc., New York, 1958 (in press).

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# ON THE PATHOGENICITY AND VIRULENCE OF THREE LABORATORY STRAINS OF *ENTAMOEBA HISTOLYTICA*

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The more than thirty-year old discussion regarding the existence or not of stable apathogenic strains of *E. histolytica* has not yet been completely settled. Neal and Vincent (1956) showed that it is possible to distinguish the stable apathogenic strains from those pathogenic ones in a temporarily avirulent phase, by injecting their trophozoites in the liver of *Cricetus auratus,* then isolating them in cultures and finally observing their action in infecting intestinally young white rats.

In my opinion, this method is justified by the following facts. It has been known for a long time (Dobell and Laidlaw, 1926) that pathogenic strains of *E. histolytica* cultivated *in vitro* in the presence of bacteria and rice starch became used to a saprophytic type of nutrition to the point of losing, with different speeds (Thompson *et al.*, 1954; Neal, 1957; de Carneri, 1958), their virulence towards the intestine of laboratory animals. This saprophytyc life and, in consequence, this involution probably correspond to that of the minuta forms in the intestinal lumen of naturally resistant carriers or those whose diet is unsuitable for the appearance of intestinal amebic lesions (Elsdon-Dew, 1956).

On the other hand, Reinertson and Thompson (1951) found that the golden hamster, more than any other animal tested, allows a good development of hepatic amebic abscesses. Thompson *et al.* (1954) also demonstrated that attenuated strains regained their invasive power towards rats' intestine after some passages through the liver of *Cricetus auratus*. Evidently, when the temporarily avirulent

trophozoites belonging to genetically pathogenic strains are faced with the necessity of surviving as intratissular parasites following injection into the liver, they resist the infested host's reaction and secrete proteolytic enzymes which allow them to nourish themselves with the products of tissular digestion, assuming the magna form. The virulent state so acquired is maintained for a sufficiently long period during successive subcultures, and this allows determination of the true pathogenicity of the strain to the intestinal walls of laboratory animal whose receptivity is similar to that of man. On the other hand, the apathogenic strains, i.e., those genetically incapable of becoming virulent towards the intestinal walls — which represent the only natural route for *E. histolytica* to enter the tissues — succumb in the golden hamster liver. In actual fact, they sometimes succeed in multiplying, producing mixed amebic and bacterial hepatic abscesses, but, once cultured and injected into the cecum, in a group of rats, they never give an average degree of infection higher than the previous (for the evaluation procedure see Jones, 1946).

It would be interesting to prolong over a period of years the *in vitro* culturing of trophozoites in the minuta form which have lost their primitive virulence, in order to check if this latter can always be reacquired or if a mutation occurs at a certain moment, depriving the ameba of the possibility of assuming the magna form parasitic to the tissues. Even without mutations, as Hoare (1952*a*) points out, *Dauermodifikationen* would also lead to racial differentiation between protozoa. This evolution towards a minor degree of pathogenic power is not only in agreement with the general evolution of parasites, but appears to be particularly advantageous for *E. histolytica,* whose magna form, which never produces cysts (Reichenow, 1953, p. 698) on multiplying in the tissues, finds itself, as far as regards the possibility of transmission to other hosts, in a real cul-de-sac (Hoare, 1952).

In an attempt to define the origin of stable apathogenic strains from initially virulent ones, I have studied the variations in virulence of 3 strains of *E. histolytica* isolated some years ago from persons suffering from various forms of amebiasis, and since then kept in the laboratory in *in vitro* cultures.

## STRAIN F22

This strain was isolated in New Orleans, during 1947, from a cerebral amebic abscess (Swartzwelder and McGill, 1949).

It has been cultivated in various media. In May 1955 it was kindly sent me by Prof. Buonomini of Pisa University and since then has been cultured in the presence of an unidentified bacterial flora on Pavlova (1938) monophasic medium. In the spring of 1955 its virulence towards the rat, tested by the method of Jones (1946), was as follows: 0 — 0 — 0 — 0 — 2 — 0 — 2 — 0 — 0 — 0 — 0 — 0 — 0. Average degree of infection = 0.3. After a further two years, in May 1957, the test was repeated, giving an A.D.I. of 0.2.

Strain F22 was then passed 9 times through the liver of *Cricetus auratus* (see Reinertson and Thompson, 1951). Unlike the observation of DeLamater *et al.* (1954), I observed that the mixed bacterial and amebic abscesses grew to fair sizes, weighing 50 mg to 200 mg. Passage from one hamster to another was always carried out directly, enrichment by culturing between one passage and the next being not necessary. At the end, the strain was returned to culture. After this, rats were infested in the cecum, with the following results: 3 — 2 — 2 — 4 — 4 — 5 — 4 — 1 — 4 — 4 — 5. A.D.I. = 3.45. Thus, after 10 years of *in vitro* cultures and after 2 years of controlled avirulence, and actually probably even more, strain F22 still transformed itself from a saprophyte into a virulent pathogenic agent.

This fact was confirmed in July 1958; at this time the avirulent branch of the F22 strain became virulent after two liver passages, giving the following results in rat's coecum: 3 — 2 — 5 — 0 — 5 — 5 — 4 — 4 — 4 — 4 — 4 — 1 — 4 — 4 — 4. A.D.I. = 3.53.

In this manner a total lifetime of 5 days in the hamster's liver was sufficient to restore the invasive properties of this strain after about 11 years of *in vitro* culture.

## STRAIN MEAH

Part of the evolution of the virulence shown by this strain has been described elsewhere (de Carneri, 1958) and is merely summarized here. Other studies are now in progress as this strain is at

present used for chemotherapeutic researches. The Meah strain was isolated by Dr. J. F. Ryley, Imperial Chemical (P) Ltd., Morley, England, at the Liverpool School of Tropical Medicine, during May 1955, from cysts passed by a patient. Associated with an unidentified bacterial flora it was kindly sent me in August 1956. In my laboratory, it is subcultured twice weekly in Pavlova medium. During 102 days the A.D.I. determined on groups of 6-12 rats descended progressively from 4.25 to 3.2 — 3.14 — 3.33 — 2.57 — 1 — 2.33 — 1.5 to 1.2. At this point, 4 passages into hamster livers were performed. The hepatic lesions produced by this strain are particularly severe, weighing from 100-600 mg. The bacteria present are generally few in number. Passage from one hamster to the next was always performed directly. In a group of 13 rats, the Meah 4 substrain gave an A.D.I. of 1.63. The series of passages in the liver were continued up to a total of 13. The substrain Meah 13 gave the following values in a group of rats: 5 — 5 — 5 — 5 — 5 — 5 — 3 — 5 — 5 — 4 — 5 — 2 — 5 — 4 — 2 — 3. A.D.I. = 4.25. This substrain was then cultured *in vitro* three times weekly.

In fact, I feared that subculturing only twice weekly, during the 4 days between each passage, the possible biotypes of more rapid metabolism — presumably the most virulent ones — might die, favouring a selection of biotypes of slower development among the amebic population. I have also stated elsewhere (de Carneri 1958a) that the trophozoites of this strain survive at most for 5 to 6 days. But the virulence diminished more rapidly in Meah 13 (2 units per month) than in the Meah strain (3 units in 3 months). In fact, during 56 days, the A.D.I. descended from 4.25 to 3.3, 2.27 to 1.75, the separate values of the latter being: 4 — 4 — 0 — 0 — 3 — 3 — 0 — 0. At this point, a single trophozoite was separated from the colony and a clonal culture prepared with the same. By this means I wished to determine if the Meah strain perhaps consisted of two main biotypes, one pathogenic, predominating after passage through the liver, and the other saprophytic, predominating after *in vitro* culture. The virulence of this clonal substrain towards the rat was not much different than the last determined for the substrain Meah 13: 4 — 0 — 4 — 0 — 3 — 2 — 4 — 0 — 3 — 3 — 5 — 0 — 0 — 4 — 3. A.D.I. = 2.33. Trophozoites were passed directly from a cecal lesion of one of these rats into a hamster liver. After isolation in Pavlova medium, the strain

was used for infecting the cecum of rats, with the following results: 5 — 3 — 5 — 5 — 4 — 4 — 4 — 0 — 1 — 4 — 3 — 0 — 2 — 4 — 3. A.D.I. = 3.13. This substrain was therefore abandoned. The original Meah strain, always subcultured twice weekly, in Pavlova medium, gave the following results in rats, during November 1957: 0— 0 — 2 — 0 — 1 — 0 — 0 — 0 — 1 — 0. A.D.I. = 0.4. During the same month, after passage through the livers of five hamsters, this same strain gave the following result in rats: 5 — 5 — 4 — 4 — 1 — 4 — 5 — 5 — 4 — 3. A.D.I. = 4. This substrain was then abandoned for culture difficulties produced by the associated bacteria. In the middle of January 1958, the original Meah strain was passed through five hamster livers, after which it gave the following results in rats: 4 — 5 — 5 — 4 — 5 — 5 — 4 — 4 — 4 — 4 — 4. A.D.I. = 4.36. After a further 42 day's culture, at the end of February, this strain had already lost part of its virulence: 0 — 4 — 4 — 2 — 2 — 5 — 4 — 2 — 3 — 0 — 0 — 5. A.D.I. = 2.6.

It may be concluded that this strain rapidly loses and reacquires its virulence and has not as yet given rise to apathogenic substrains.

## STRAIN EDM

As related to me by Prof. L. Lamy, Pasteur Institute, Paris, who kindly sent me this strain in September 1954, it was isolated in Paris, during 1946, from an individual suffering from chronic amebic colitis. In the presence of mixed bacterial flora, it grows well in the above-mentioned medium. In spring 1955 it gave the following results in rats: 1 — 0 — 0 — 0 — 0 — 0 — 0 — 0 — 1 — 0 — 1 — 0. A.D.I. = 0.25. In June 1957 a first attempt was made to make the strain virulent by passages through hamster liver. The hepatic lesions were small; quite often the trophozoites were not visible on direct microscopic examination. Using a total of 28 hamsters, it was not possible to proceed further than the fourth passage and as thin strain did not grow well *in vitro,* it was abandoned. The attempt was again made in 1957 — December — after a second control of strain EdM in rat had given an equally low A.D.I. The strain was 6 times passed through hamster liver; this time it was possible to carry out all 6 passages directly, without having recourse to intermediate enrichment in culture. The

substrain EdM 6 was then isolated, which gave the following results in rats: 1 — 0 — 0 — 0 — 0 — 0 — 2 — 3 — 2 — 1 — 1 — 0 — 0 A.D.I. = 0.77. As 1 rat in 13 showed amebic lesions, although only microscopically, the substrain was immediately passed a further 5 times through hamster liver, without any difficulty. At the end, the following results in rats were obtained: 0 — 0 — 0 — 0 — 2 — 0 — 0 — 4 — 3 — 0 — 2 — 1. A.D.I. = 1. This time, 1 out of 12 rats had a microscopic lesion and another a macroscopic amebic lesion. The test was repeated the following week with these results: 0 — 2 — 0 — 0 — 1 — 0 — 0 — 0 — 0 — 0 — 1 — 0 — 0 — 3 — 0 — 1. A.D.I. = 0.5. Only 1 rat in 16 demonstrated microscopic lesions on the cecal walls. Unfortunately, attempts to isolate a culture from these lesions failed. I think it impossible to obtain any further increase by performing more passages in liver. In fact, there is no positive difference between the A.D.I. after 6 passages and that after II, on comparing the average figures. On the whole, the A.D.I. reached remains too low to consider the strain as truly virulent (see Neal, 1957, criticizing an estimation made by Beaver, 1956). These amebae were however capable of growing in the intestinal walls of 11 % of those animals we used for the test because their average receptivity is similar to that of man. To give a comparison, this percentage is more than 8 times greater than that of cases of amebic dysentery among *E. histolytica* carriers in an endemic focus in Armenia (Sarkisyan, 1955) and more than 30 times greater than the figures for S. Domingo (Westphal, 1949), i. e., for regions where this disease has a particularly severe incidence.

## SUMMARY AND CONCLUSION

Three strains of *Entamoeba histolytica* isolated respectively in Paris, during 1946, from a case of chronic colitis (strain EdM); in New Orleans, during 1947, from a brain abscess (strain F22) and in Liverpool, during 1955, from cysts expelled by a patient (Meah strain) at the Liverpool School of Tropical Medicine, and since then cultured in the laboratory in the presence of a mixed bacterial flora, were found to be avirulent when injected into the cecum of young white rats throughout the period 1955-1957. However, after a series of passages through the liver of *Cricetus auratus* the last two strains re-acquired high virulence towards the intestinal walls of the rat. After passage through liver, strain EdM re-acquired a rather modest virulence if compared with that of the other two strains and with still other pathogenic ones, as seen from the literature. However, even this strain

was, in the end, capable of invading the walls of the cecum in 11 % of the injected rats. It is concluded that even artificial segregation in culture tubes for a period of 3 to 12 years, which allows growth of *E. histolytica* under strictly saprophytic conditions, does not favour the appearance of a mutation definitively depriving this protozoan of its pathogenicity, i.e., of the possibility of invading the intestinal walls in certain circumstances. If a similar transformation occurs in nature, it must be rather rare. Furthermore, this is obvious from the present existence of virulent strains after thousands of years of living in man, which naturally favours the selection of less virulent and more regular cyst-producing strains.

*N. B.* — After this communication was sent off in April 1958, studies have been extended to strain P coming from a case of amebic dysentery and isolated by Dobell in monoxenous culture with *Escherichia coli,* strain A' (see Dobell, Neal, Hoare, 1952). This ameba has lost all virulence for the rat; nevertheless, after association with mixed bacteria deriving from the Meah strain it was found to display slight virulence (A.D.I. = 1.77), mildly ulcerating 23 % of the rats. Passage through 4, 8 and 12 hamsters did not further increase the virulence. It appears possible to conclude that of the strains studied the least pathogenetic are those which have remained longest in culture. After 3 years, the Meah strain has a maximum virulence of 4.36, after 11 years, strain F22's maximum virulence is 3.53, after 12 years, strain EdM's is 1, whilst after 13 years of monoxenous culture, strain P's is 1.77. Further experiments are necessary to definitively confirm that not only may the virulence weaken, but even the pathogenic properties of an *E. histolytica* population evolve and disappear, although more slowly, whenever this protozoan lives for years as a saprophyte, i.e., as a commensal. The method used for the present studies might finally give a satisfactory response to this important question (see de Carneri, 1958*b*).

## BIBLIOGRAPHY


Beaver, P. C., Jung, R. C., Sherman, H. J., Read, T. R., and Robinson, T. A. — «Experimental *Entamoeba histolytica* infections in man», *Am. J. Trop. Med. & Hyg.,* **5**: 1000-1009, 1956.

de Carneri, I. — «Studi sulla virulenza di *Entamoeba histolytica.* I. Variazioni cicliche della virulenza di un ceppo di *Entamoeba histolytica* nell'infezione intestinale del ratto albino in seguito a passaggi seriali in vitro e nel fegato di *Cricetus auratus*», *Riv. Parassitol.,* **19**: 7-20, 1958.

de Carneri, I. — «The *in vitro* sensitivity of four strains of *Entamoeba histolytica* after various periods of incubation», *Arch. int. pharmacodyn.,* **113**: 273-289, 1958*a*.

de Carneri, I. — «Recherches sur l'origine possible des souches apathogènes de *Entamoeba histolytica*», *Bull. Soc. Path. exot.,* **51**: 616-627, 1958*b*.

DeLamater, J. N., Michaelson, J. B., Hallman, F. A., and Blumenthal, H. — «An investigation into hyaluronidase as a factor in the mechanism of tissue invasion by *Endamoeba histolytica*», *Am. J. Trop. Med. & Hyg.*, **3**: 1-8, 1954.

Dobell, C., and Laidlaw, P. P. — «On the cultivation of *Entamoeba histolytica* and some other entozoic amoebae», *Parasitology*, **18**: 283-318, 1926.

Dobell, C., completed by Neal, R. A., edited by Hoare, C. A. — «Researches on the intestinal protozoa of monkeys and man. XII. Bacterial factors influencing the life history of *Entamoeba histolytica* in cultures», *Parasitology*, **42**: 16-39, 1952.

Elsdon-Dew, R. — «Further aspects of amoebiasis in Africans», *Centr. Afr. J. Med.*, **2**: 291-294, 1956.

Hoare, C. A. — «The commensal phase of *Entamoeba histolytica*», *Exper. Parasitol.*, **1**: 411-427, 1952.

Hoare, C. A. — «The taxonomic status of biological races in parasitic protozoa», *Proc. Linn. Soc. London., Sess. 163*, Pt. 1, 44, 1952*a*.

Jones, W. R. — «The experimental infection of rats with *Entamoeba histolytica;* with a method for evaluating the anti-amoebic properties of new compounds», *Ann. Trop. Med.*, **40**: 130-140, 1946.

Neal, R. A., and Vincent, P. — «Strain variation in *Entamoeba histolytica*. II. The effect of serial liver passage on the virulence», *Parasitology*, **46**: 173-182, 1956.

Neal, R. A. — «III. Virulence in *Entamoeba histolytica*», *Trans. Roy. Soc. Trop. Med. Hyg.*, **51**: 313-319, 1957.

Pavlova, E. A. — «Sur les méthodes de la culture d'*Entamoeba histolytica*», *Med. Parazitol.*, Moscow, **7**: 244-277, 1938.

Reichenow, E., in Doflein, F., and Reichenow, E. — *Lehrbuch der Protozoenkunde*, sixth ed., Jena, 1953.

Reinertson, J. W., and Thompson, P. E. — «Experimental amebic hepatitis in hamsters», *Proc. Soc. Exper. Biol. Med.*, **76**: 518-521, 1951.

Sarkisyan, M. A. — «Amebiasis morbidity and the carriage of *Entamoeba histolytica* in an endemic focus», *Med. Parazitol.*, Moscow, **24**: 311-315, 1955.

Swartzwelder, J. C., and McGill, H. C. — «Intestinal amebiasis complicated by abscess of the brain», *Rev. Kuba de Med. Trop.*, **5**: 27-29, 1949.

Thompson, P. E., McCarthy, D., and Reinertson, J. W. — «Observations on the virulence of *Endamoeba histolytica* during prolonged subcultivation», *Am. J. Hyg.*, **59**: 249-261, 1954.

Westphal, A. — «Amöbenruhr auf Santo Domingo», *Z. Tropenmed. Parasitol.*, **1**: 225-230, 1949.

# THE EPIDEMIOLOGY OF INTESTINAL PROTOZOA IN FAMILY GROUPS OF FOUR JAPANESE VILLAGES [1]

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For both epidemic and endemic amebiasis, the relative importance of the various modes of transmission are still strictly conjectural. For the few epidemics studied, water has been implicated as the mode of transmission more often than all other known means combined. However, information from such studies may not be applicable to endemic conditions, the latter being more difficult to judge since several means of exposure may occur simultaneously and each may vary in importance between different parts of the world or even communities of the same area.

Familial amebiasis has been noted repeatedly and assumed to stem from intrafamilial transmission, with foodhandlers and direct contacts with individuals and fomites as corollary means of exposure. Endemic transmission by water, an epidemiologic influence common to all members of a household, has received limited support. Living habits and environmental conditions have been categorized, predicting a correlation between fecal contamination and occurrence of protozoan infections (Eyles *et al.*, 1953; Mackie *et al.*, 1956).

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The present work is concerned with the correlation of protozoan infections with known environmental conditions and family relations in a series of family groups selected from a rural area of Yamanashi Prefecture, Honshu, Japan.

## METHODS

Four farming communities in the Kofu valley cooperated in the project. The primary valley comprises about 100 square miles, linked with numerous secondary valleys of the enclosing mountains where two of the villages are located. Five stools over a 10 day period were requested from each of 1,037 persons from 172 families, an average of 4.94 per person being submitted. The stools were collected at home and examined in a nearby laboratory using the formalin-ether concentration procedure (Ritchie, 1948; Wykoff *et al.*, 1958). A family sheet was prepared for basic information: family and individual number, age, sex, family relationship, foodhandler status (primary or secondary), and results of the examination.

Sources of drinking water for the four villages differed considerably. Village M (Mutsusawa), located on a secondary valley where well digging was not possible, obtained water from an irrigation canal which also served for washing night soil buckets, clothes and vegetables. Village T (Tatomi), on the flat of the main valley, obtained water from ground-level troughs filled from pipes driven into the ground (continuous flow). In village H (Hikawa) wells were in use, some open, some sealed (with pumps). Village K (Kissawa), located in a secondary valley, used wells, but the steep terrain increased the hazard of run off water entering them.

No significant variations were observed between villages for their toilet facilities, means of sewage and garbage disposal, or household sanitation.

## RESULTS

*Parasitic incidence.* — One or more parasites were found in 96.8 % of the individuals examined, 96.6 % had one or more helminthic

infections. One or more protozoa were harbored by 63.5 %. The incidence of *Entamoeba histolytica* was 21 %; for other protozoa, *E. coli*, 50 %, *Endolimax nana*, 35 %, *Iodamoeba bütschlii*, 6 %, *Giardia lamblia*, 13 %. For villages M, T, H, and K, respectively, the occurrence of *E. histolytica* was 29, 14, 17, and 23 %. *E. coli* ranged from 43 % to 56 %, and *E. nana* from 33 % to 39 % (Table 1).

TABLE 1 — *Percentage incidence of protozoan infections in 4 rural Japanese villages among individuals and family groups*

| Infections | Village M | Village T | Village H | Village K | Totals |
|---|---|---|---|---|---|
| | POPULATION INCIDENCE | | | | |
| | Number of persons | | | | |
| | 308 | 229 | 251 | 249 | 1,037 |
| *E. histolytica* | 29 | 14 | 17 | 23 | 21 |
| *E. coly* | 49 | 43 | 52 | 56 | 50 |
| *E. nana* | 33 | 34 | 39 | 35 | 35 |
| *E. bütschlii* | 10 | 1 | 6 | 6 | 6 |
| *G. lamblia* | 17 | 11 | 6 | 16 | 13 |
| | FAMILY INCIDENCE [1] | | | | |
| *E. histolytica* | 84 | 54 | 75 | 61 | 69 |
| *E. coli* | 98 | 90 | 100 | 94 | 95 |
| *E. nana* | 89 | 85 | 93 | 85 | 88 |
| *E. bütschlii* | 42 | 7 | 33 | 24 | 27 |
| *G. lamblia* | 67 | 42 | 28 | 54 | 48 |

[1] One or more protozoa in at least one member of a family.

The family incidence of *E. histolytica* (one or more infections per family) was 69 % collectively, and for the four communities: 84, 54, 75, and 61 %. Between 90 and 100 % of the families in each village had at least one infection of *E. coli*, the overall figure being 95 %; correspondingly, for *E. nana*, 85 to 93 %, and 88 % collectively.

Only 2 of the 172 families were completely free of intestinal protozoa, and 6 had only one infection each.

*The role of the mother (foodhandler) in intrafamilial transmission.* — As the mother of the household is commonly the primary foodhandler and because of her intimacy with each family member, an

TABLE 2 — *The occurrence of specific protozoa in family groups when the mother, father or a member selected by chance* was infected

| | | Mother | Father | Chance selectee | Total |
|---|---|---|---|---|---|
| *E. histolytica* | Number of families | 64 | 42 | 32 | 138 |
| | Total members | 398 | 268 | 209 | 875 |
| | Number infected | 145 | 94 | 80 | 319 |
| | Percent infected | 36.4 | 35.1 | 38.3 | 36.4 |
| | Chi square | 0.001 | 0.221 | 0.298 | 0.519* |
| *E. coli* | Number of families | 113 | 85 | 81 | 279 |
| | Total members | 678 | 540 | 509 | 1,727 |
| | Number infected | 403 | 330 | 331 | 1,064 |
| | Percent infected | 59.4 | 61.1 | 65.0 | 61.7 |
| | Chi square | 1.349 | 0.057 | 2.518 | 3.924* |
| *E. nana* | Number of families | 94 | 62 | 58 | 214 |
| | Total members | 574 | 391 | 352 | 1,317 |
| | Number infected | 259 | 190 | 172 | 621 |
| | Percent infected | 45.1 | 48.6 | 48.9 | 47.2 |
| | Chi square | 0.951 | 0.325 | 0.413 | 1.689* |

* For two degrees of freedom.
P = 0.05 when chi square = 5.991.
P = 0.01 when chi square = 9.210.

attempt was made to determine whether there was a linkage of familial infections with infected mothers. A correlation was made between protozoan rates in families where the mother was positive, and, for comparison, where the father was positive (Table 2). For further comparison, a member of the household was selected at random and these members, when positive, were correlated with incidence within their families. The rates for positive mothers, fathers and chance

selectees were, respectively, for *E. histolytica,* 36, 35, and 38 %; for *E. coli,* 59, 61, and 65 %; and *E. nana,* 45, 49, and 49 %. Differences involved were minimal and, with one exception, without statistical significance.

Another correlation with even better defined categories was made to further test the possible role of the mother in intrafamilial transmission. Families were selected in which the mother was the only adult infected with *E. histolytica.* Of 34 such families, 23 (19 %) of the 129 children were found to harbor this parasite. Similarly, when the father was the only adult member infected with this parasite, 12 (15 %) of the 80 children from such families harbored *E. histolytica.* The differences are without statistical signifance.

A total of 58 families were noted in which no adult member was infected with *E. histolytica.* Of the 204 children in such families, 21 (10 %) harbored this parasite.

*Seasonal variations in protozoan infections.* — In order to serve as a comparison of intestinal protozoa in summer and winter, individuals in villages H and K were examined 5 times in both January and early September. For the former village the incidence of *E. histolytica* was 17 and 21 % in January and September, respectively, while in village K the corresponding figures were 23 and 19 %. For other protozoa, corresponding differences were limited to 3 % or less.

*Age and sex correlation.* — Among the 1,037 individuals, females were in predominance for the 4 protozoan infections. To further validate these observations on *E. histolytica,* data from the present study were combined with those previously obtained in 18 other villages in this same valley area (Wykoff *et al.,* 1955). The greatest sex-incidence difference in favor of females occurred in the 16-20 and 41-50 year groups, the differences being statistically significant. Disregarding age, the females had a significantly greater overall incidence than males. *Entamoeba histolytica* and *E. nana* first occurred at near maximum levels between 16-20 years and *E. coli* between 11-15 years. All showed slight increases throughout adulthood, except for a decline over 60 years, particularly for *E. histolytica* (Fig. 1).

*Correlation of family size and occurrence of protozoan infections.* — Data from the present study were correlated so as to reveal whether overcrowding in the homes (as reflected by family size) could

account for increased transmission through direct contact. For families of size 2-5, 6-7, and 8-10, the incidence of *E. histolytica* was found to be 20, 20, and 25 %, respectively. Likewise, for *E. coli* the incidences

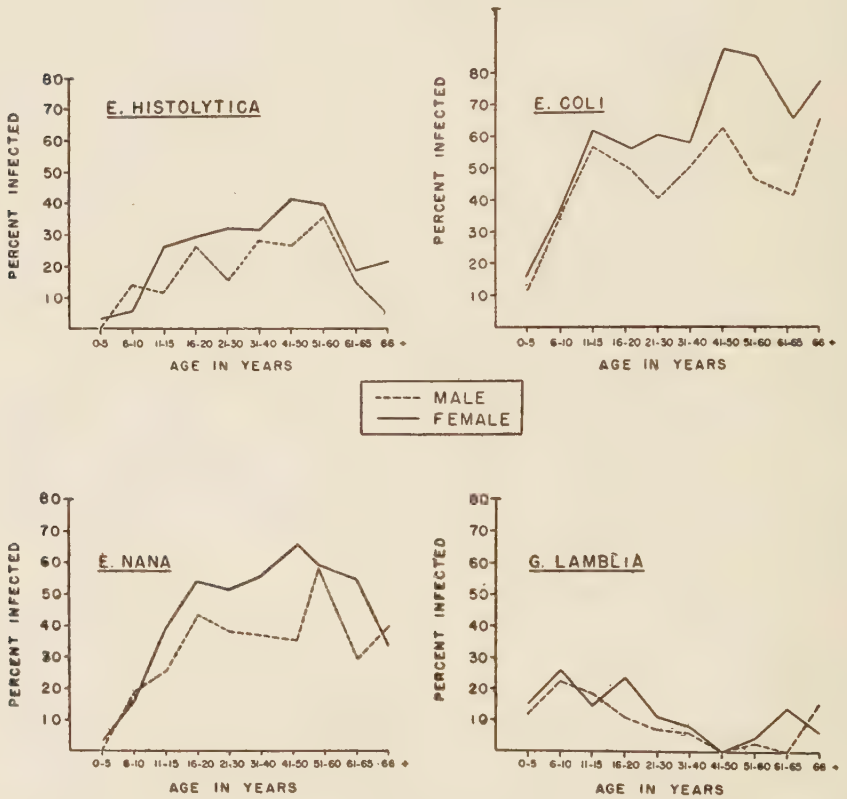


Fig. 1 — Age and sex incidence of intestinal protozoa in family groups of 4 rural Japanese villages

were 48, 49, and 53 %. A trend of higher incidence for larger families may be noted, but credence is hardly warranted because differences are minimal (Table 3).

TABLE 3 — *Correlation of family size and occurrence of protozoan infections in 4 rural Japanese villages*

| | Family size (number of members) | | | | | |
|---|---|---|---|---|---|---|
| | 2–5 | | 6–7 | | 8–10 | |
| Number of families | 56 | | 78 | | 38 | |
| Total of persons | 253 | | 506 | | 324 | |
| Infections | N.° | % | N.° | % | N.° | % |
| *E. histolytica* | 50 | 20 | 102 | 20 | 82 | 25 |
| *E. coli* | 122 | 48 | 249 | 49 | 173 | 53 |
| *E. nana* | 86 | 34 | 184 | 36 | 115 | 36 |
| *I. bütschlii* | 14 | 6 | 24 | 5 | 22 | 7 |
| *G. lamblia* | 30 | 12 | 63 | 13 | 43 | 13 |

## DISCUSSION

The degree of linkage of infections within households appeared to be considerably less in this Japanese population than in those studied in the U.S.A. (Mackie *et al.*, 1955; Eyles *et al.*, 1953). The present data fail to link the infected mother with incidence of *E. histolytica, E. coli* or *E. nana* in her family. There was an equal or greater relationship between the incidence of the father (a non foodhandler) and his children. When a positive individual was selected by chance from each family, the correlation with protozoan infections in other family members was greater than when either the infected mother or father was used as the proband. Likewise, in contrast with findings in the U.S.A., the present work has not established a relationship between infected adults and children within the same family. This was due, at least in part, to the fact that the infection level for children was below that of adults.

It was reported (Eyles *et al.*, 1953) that crowding within the home appeared to increase transmission by direct contact. However,

a bias existed in their data correlations (noted by Brooke *et al.*, 1955) and revised figures were not supportative. It appears that correlation of incidence with family size is of doubtful value in evaluating the relative importance of different means of exposure in this Japanese group.

It has been reported that exposure to intestinal protozoa is linked with exposure to fecal wastes, and thus most of rural Japan should have high rates. This is apparently not the case. The present 4 villages were selected because of their high rates, but the incidence of *E. histolytica* in 18 villages on the valley floor ranged from 0 to 10 % on the basis of a single fecal examination (Wykoff *et al.*, 1955).

The current study indicates higher rates of *E. histolytica* in females than in males. The possibility of women eating precooked tidbits which are commonly washed in streams might account in part for these higher rates.

Considerable variation in the age-incidence pattern for intestinal protozoa of different populations have been observed (Faust, 1930; Eyles and Jones, 1954; Jones *et al.*, 1954). Concerning the acquisition rate and earliest age of near-maximum incidence, Eyles *et al.* (1953) postulated that maximum acquisition during childhood, followed by a decline in adulthood, could be best explained by direct contact with infected persons and fomites. An alternate conjecture appears indicated: that the earliest age at which near maximum levels prevail may be a corollary of overall prevalence or the exposure hazard it reflects, rapid acquisition being associated with high incidence and vice versa. When sanitation is poor, rapid acquisition and a near-maximum incidence within 5-10 years might be expected, with minimal increases thereafter. Conversely, good sanitation would minimize contraction rate with maximum levels being delayed until young adulthood, followed by a decline due to spontaneous loss of infections exceeding new infections, as assumed by Eyles *et al.* (1953) and demonstrated by Beaver *et al.* (1956).

The epidemiologic picture embraces certain factors which are seemingly uniform throughout rural Japan. There was a low level of sanitation with correlated potentials for transmission by foodhandlers and direct contacts with infected persons and fomites. Flies were abundant with toilets and night soil storage tanks available as breeding sites nearby. Practices of handling night soil and general habits of

living were everywhere similar, yet the incidence of protozoa varied among the villages. The factor most variable was the domestic water supply. The 4 villages studied represented a transition from hilly to valley terrain, for which there appeared to be a related decline in incidence of *E. histolytica* (Wykoff *et al.*, 1955).

In the past, foodhandlers and direct contacts with infected persons have been considered as means of intrafamilial transmission. It appears that contaminated water supplies might also account for such linkage. If the Mantetsu water outbreak of amebiasis in Japan (Ritchie and Davis, 1948) had been overlooked, family groups infected there would have constituted classical examples of familial linkage of protozoan infections upon return to communities in the U.S.A.

It is thus proposed that combined influence of food handling, direct contact with infected persons, family size, and seasonal variations do not constitute *primary* means for transmission of protozoa in the population studied. It appears likely that variations in the relative importance of the several modes of transmission may be linked with the level of infection, e.g., where the incidence is high, as in the present study, water may be the predominant means of transmission, whereas other factors may be relatively more important under moderate or low endemicity.

## SUMMARY

The endemicity of intestinal protozoa was studied in 172 family groups of 4 rural villages of the Kofu valley, Japan. Only 2 of these families were completely free of protozoa, incidence by family for *E. histolytica, E. coli,* and *E. nana* being 69, 95, and 88 %, respectively. Among 1,073 persons the corresponding frequencies were 21, 50, and 35 %. Among the 4 villages, the highest rate in individuals was 29 %.

No seasonal influence was noted; females had significantly higher rates of infection, and near-maximum incidence for *E. histolytica* and *E. nana* occurred between 16-20 years. The data did not reveal that infections in a family were particularly associated with one individual more than another. Family size and resulting crowding did not appear to be an epidemiological influence of any consequence.

Community-wide transmission was indicated, overshadowing intrafamilial transmission, to whatever extent it occurred. The epidemiological picture embraced a series of factors which appeared uniform for rural Japan (sanitation, personal hygiene, fly-prevalence, practices of handling night soil, and general living habits) which could not account for the variation of protozoa from one village to another.

The water supply, however, was a variable related to incidence. It is proposed that where incidence is high as in the present study, that water may be the predominant means of transmission, whereas other factors may be relatively more important in areas of moderate or low endemicity.

## BIBLIOGRAPHY


BEAVER, P. C., JUNG, R. C., SHERMAN, H. J., READ, T. R., and ROBINSON, T. A. — «Experimental *Entamoeba histolytica* infections in man», *Am. J. Trop. Med. and Hyg.*, **5**: 1000-1009, 1956.

BROOKE, M. M., MELVIN, D. M., SAPPENFIELD, R., PAYNE, F., CARTER, F. R. N., OFFUTT, A. C., and FRYE, W. W. — «Studies on a water-borne outbreak of amebiasis, South Bend, Indiana. III. Investigation of family contacts», *Am. J. Hyg.*, **62**: 214-226, 1955.

EYLES, D. E., JONES, F. E., and SMITH, C. S. — «A study of *Endamoeba histolytica* and other intestinal parasites in a rural West Tennessee community», *Am. J. Trop. Med. and Hyg.*, **2**: 173-190, 1953.

EYLES, D. E., and JONES, F. E. — «The prevalence of *Endamoeba histolytica* and other intestinal parasites in a selected urban area», *Am. J. Trop. Med. and Hyg.*, **3**: 988-994, 1954.

FAUST, E. C. — «A study of the intestinal protozoa of a representative sampling of the population of Wise County, southwestern Virginia», *Am. J. Hyg.*, **11**: 371-384, 1930.

JONES, F. E., SMITH, C. S., and EYLES, D. E. — «Epidemiological study of *Endamoeba histolytica* and other intestinal parasites in the New Hope community of Tennessee (a restudy after 21 years)», *Am. J. Trop. Med. and Hyg.*, **3**: 266-275, 1954.

MACKIE, T. T., MACKIE, J. W., VAUGHN, C. M., GLEASON, N. N., GREENBERG, B. G., NENNINGER, E. S., LUNDE, M. N., MOORE, L. L. A., KLUTTZ, J. A., and TALIAFERO, M. O. — «Intestinal parasitic infections in Forsyth County, North Carolina. III. Amebiasis in school children, an index of prevalence», *Am. J. Trop. Med. and Hyg.*, **4**: 980-988, 1955.

MACKIE, T. T. — «Intestinal parasitic infections in Forsyth County, North Carolina. IV. Domestic environmental sanitation and the prevalence of *Entamoeba histolytica*», *Am. J. Trop. Med. and Hyg.*, **5**: 29-39, 1956.

RITCHIE, L. S. — «An ether sedimentation technique for routine stool examinations», *Bull. U. S. Army Dept.*, **8**: 326, 1948.

RITCHIE, L. S., and DAVIS, C. — «Parasitological findings and epidemiological aspects of epidemic amebiasis occurring in occupants of the Mantetsu apartment building, Tokyo, Japan», *Am. J. Trop. Med.*, **28**: 803-816, 1948.

WYKOFF, D. E., FONSECA, J. R. C., and RITCHIE, L. S. — «Epidemiology of amebiasis: possible influence of water supply, coincident with diverse features of terrain, on the occurrence of intestinal protozoa», *Am. J. Trop. Med. and Hyg.*, **4**: 465-471, 1955.

WYKOFF, D. E., FRICK, L. P., and RITCHIE, L. S. — «Statistical evaluation of the formalin-ether (406th MGL) fecal sedimentation concentration procedure», *Am. J. Trop. Med. and Hyg.*, **7**: 150-157; 1958.

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# ASPECTOS CLÍNICOS-EXPERIMENTALES Y FARMACOLÓGICOS DEL 11925 Y SU ACTIVIDAD AMIBICIDA

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## INTRODUCCIÓN

Todo tratamiento debe cumplir con un doble propósito:

1) Ser amibicida;
2) Tratamiento del síndrome «amibiasis».

Es decir que la actividad farmacológica debe ir dirigida a:

1) Destruir la amiba;
2) Erradicar la «amibiasis».

Todo antiparasitario debe llenar las siguientes premisas:

1) Ser eficaz;
2) Ser inocuo;
3) Ser relativamente económico;
4) Ser más o menos agradable;
5) Estar exento de fenómenos secundarios.

La invasión de diferentes órganos y tejidos por las amibas crea aspectos clínicos que pueden ser decisivos para la elección del agente terapéutico etiotropo. En la primera fase de su evolución la afección se caracteriza por la invasión del colon y sólo secundariamente aparecen lesiones «metastásicas», permítasenos mal emplear este término, tan gráfico, en otros órganos. Significa ésto que el tratamiento precoz, eficaz e intensivo es la profilaxia más segura de los otros síndromes y complicaciones graves producidas por la amibiasis.

Es el procedimiento más simple para evitar las formas crónicas con honda repercusión sobre la vida económica de los individuos.

Por lo tanto, el tratamiento de las afecciones producidas por la entameba dysenteriae depende en gran parte de la etapa y asiento de la enfermedad.

Los amibicidas disponibles hasta 1950 satisfacen parcialmente. En numerosas oportunidades no se logra suprimir radicalmente las amibas con un solo preparado y menos curar una amibiasis verdadera, sino que debe asociar diversas substancias. Como la flora juega un papel fundamental en la capacidad patogénica de la amiba, se emplean asociaciones de amibicidas y antibacterianos.

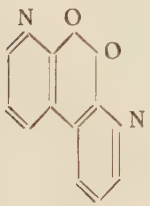
No haremos la historia de la terapéutica antiamibiana, pero diremos sólo que, hasta 1951, eran únicamente los arsenicales amibicidas directos. En ese entonces se incorpora al largo arsenal un antibiótico específico, la fumagilina.

Todo nuevo preparado contra la amibiasis será particularmente interesante, si es capaz de traer modificación de pH, rH intestinal, poseer propiedades antibacterianas y amibicidas, y además reducida toxicidad.

En Basilea, el laboratorio experimental de Ciba ha sintetizado últimamente una substancia sin parentezco químico con ninguno de los antiamibianos conocidos y cuya efectividad es lo bastante específica, experimentalmente, para inducirnos a proceder a un ensayo en el ser humano cuyos resultados expondremos en este trabajo.

Desde abril 1955 a octubre usamos el 11925 para verificar su actividad amibicida y antibacteriana. Vómitos precoces, por irritación gástrica, le hicieron agregar cubierta entérica.

Desde mayo 1956 usamos 11925 C, cuya fórmula de estructura es:




5,6-quinona-4,7-fenantrolina

Grageas con cubierta entérica — droga activa: 50 mg

## PROPIEDADES QUIMIOTERÁPICAS EXPERIMENTALES

CUADRO N.º 1 — *Acción amibicida* in vivo *cobaios, conejos, rata, gato joven*

| Dosis mg/kg *per os* | Duración del tratamiento | Número de animales | Amibas en el ciego ó en sigmoide de (gato) | | |
|---|---|---|---|---|---|
| | | | ø | + | ++ |
| 50 . . . . . | 5 días | 15 | 10 | 4 | 1 |
| 75 . . . . . | 5 días | 10 | 8 | 2 | — |
| 100 . . . . . | 5 días | 20 | 19 | 1 | — |
| Control . . . . . | 5 días | 25 | 6 | 9 | 10 |

CUADRO N.º 2 — *Acción amibicida* in vitro

| | Concentración | Acción |
|---|---|---|
| Entameba dysentérica (cultivo fresco) . . . | 1:12.000 | Amibicida total |

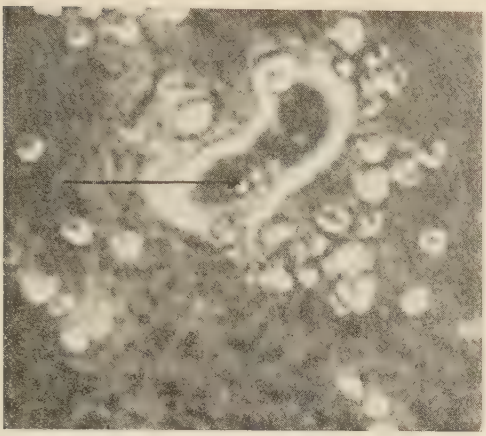


Fig. 1 — Amiba microfotografiada (microscopio de contraste de fases). Obtenida de nuestro medio de cultivo. Trofozoito de perfiles netos. Los corpúsculos que se ven a su alrededor corresponden a gránulos de almidón de 5 μ a 8 μ, necesarios para el buen desarrollo del protozoario en el medio de cultivo. Los otros elementos que rodean a la amiba son bacterias, cuyo metabolismo, en interacción con los elementos constitutivos del cultivo, permiten crear en éste las condiciones biológicas necesarias para el desarrollo del protozoario

Para el estudio de la acción *in vitro* del 11925 utilizamos amibas obtenidas de nuestro medio de cultivo, 48 a 72 horas después de realizado. Sobre un portaobjeto hacemos un extendido de la superficie del cultivo. Se ocluye con un cubreobjeto. Agregamos por capilaridad una gota de la suspensión de 11925, en una dilución de 1:12.000. Se ocluyen los bordes parafinándolos. Se incuba a 37°C durante 18-36-48-75 horas.

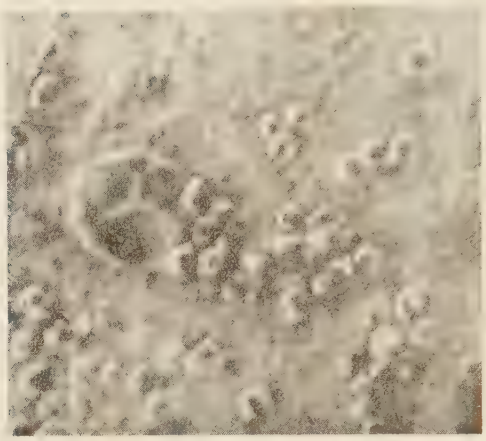


Fig. 2 — Después de 18 horas de incubación hacemos un preparado que microfotografiamos. El protozoario comienza a aumentar de tamaño. Sus bordes se hacen nítidos, pierde motilidad y aparecen las primeras vacuolas, primer indicio de la muerte citoplasmática

Fig. 3 — A las 36 horas el protozoario sigue aumentando de tamaño. El citoplasma y la membrana pierden brillo. Aumenta el tamaño de la vacuola, aumentan en número y rechazan el núcleo hacia la periferia

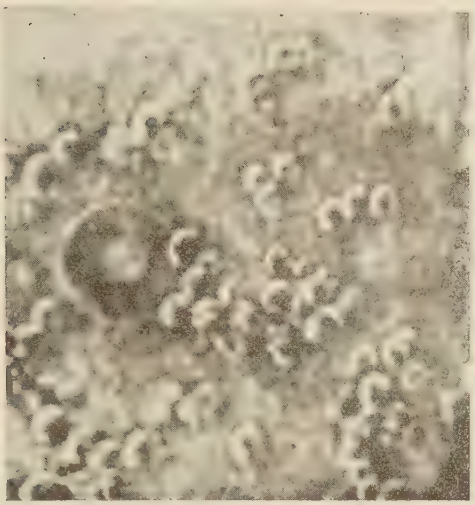


Fig. 4 — Las vacuolas que aumentan de tamaño terminan por unirse en una vacuola central, a las 48 horas. La amiba, muy agrandada, ha perdido casi por completo la motilidad

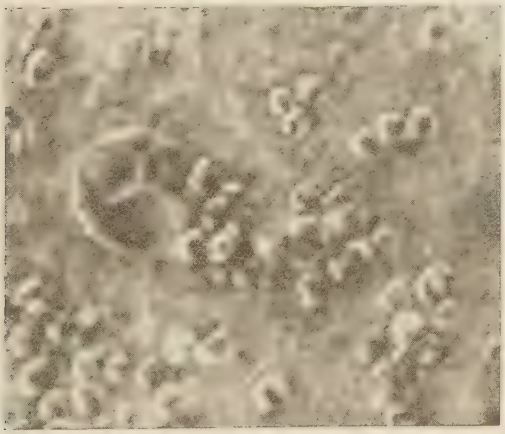


Fig. 5 — Otras amibas, a las 48 horas, comienzan su retracción y aproximan el núcleo a la periferia

Fig. 6 — A las 60 horas en algunas de las amibas estalla el núcleo y el protozoario comienza a disgregarse

Fig. 7 — A las 75 horas el protozoario ha desaparecido

CUADRO N.o 3 — *Acción antibacteriana del 11925 C*

| Cepa bacteriana | Concentraciones bacteriostáticas mínimas |
|---|---|
| *Streptococcus fecalis* | 1 : 150.000 |
| *Esch. coli* grupo 0-111 : B-4 | 1 : 16.000 |
| *Esch. coli* grupo 0-26 : B-6 | 1 : 20.000 |
| Aerobacter aerógenes | 1 : 50.000 |

## TOXICIDAD

CUADRO N.o 4 — *Toxicidad aguda* per os *del 11925 C*

| Animal | DL 50 g/kg *per os* |
|---|---|
| Rata | 3,0 |
| Conejo | 0,5 |
| Cobayo | 1,2 |
| Gato joven | 2 |

CUADRO N.o 5 — *Toxicidad crónica* per os *del 11925 C*

| Animal | Dosis diaria bien tolerada | Número de días |
|---|---|---|
| Rata | 100 mg/kg | 20 |
| Gato joven | 100 mg/kg | 30 |
| Conejo | 30 mg/kg | 25 |
| Cobayo | 75 mg/kg | 30 |

No se hallaron lesiones en la anatomía patológica.

## EXPERIENCIA HUMANA ANTERIOR A LA NUESTRA

No hay publicada.

## NUESTRA EXPERIENCIA CLÍNICA Y DE LABORATORIO

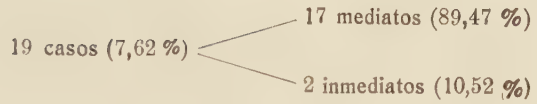
45 casos con 11925, cubierta endulcorada.
57 casos con 11925, cubierta entérica.
248 casos con 11925 C, cubierta entérica.
Todos fueron estudiados así:

- **Clínicamente**
  - Examen somático
    - Estado general
    - Peso
  - Examen de laboratorio cada 3 días
    - Orina
      - Albúmina
      - Sedimento
      - Recuento de Addis
    - Sangre
      - Hemograma
      - Tiempo de protrombina
      - Urea
      - Glucemia
      - Hanger
  - Estudio del régimen alimentario
- **Coprológicamente** controles diarios 20 días. Luego cada 15 días hasta 1 año de comenzado el tratamiento
  - Estudio funcional de la capacidad digestiva
  - Estudio bacteriológico
  - Estudio parasitológico
    - Flotación de Faust, coloración
      - Donaldson
      - D'Antoni
    - Hematoxilina (Man-Dobell)
    - Cultivo y su pasaje por inoculación al sigmoide del gato joven

## CASUÍSTICA

248 casos (100 %)
- 105 ♂ adultos (42,33 %) (15 a 79 años)
- 79 ♀ adultos (31,85 %) (15 a 66 años)
- 64 niños (25,80 %)
  - ♂ 22 (10 a 15 años)
  - ♀ 42 ( 9 a 15 años)

INTOLERANCIA




MANIFESTACIONES CLÍNICAS DE INTOLERANCIA

2 cefaleas (1 inmediata).
2 diarreas.
1 erupción petequial generalizada.
1 taquicardia paroxística.
8 hematurias microscópicas.
5 alargamiento del tiempo de protrombina.
1 dolores articulares.
1 diplopía.
3 náuseas (1 inmediata).
2 vómitos (1 incoercible).

RESULTADOS

En esta serie de 248 casos bien estudiados, con la técnica expuesta, hemos obtenido buen resultado, modificando la flora y eliminando el protozoario.

En los casos tomados para este informe se estudió a los familiares, para evitar reinfestaciones.

Las amibas desaparecen de las heces del 7.º al 15.º día en 209 casos (84,27 %).

Hasta los 20 días, 19 casos más (7,66 %). En los 20 casos restantes se repitió otra serie después de 20 días de descanso. Fracasó en 4 casos (1,61 %). 3 (1,20 %) por intolerancia de la droga (taquicardia paroxística, erupción petequial, vómitos incoercibles, todas con la 2.ª serie) y el restante una cepa amibiana irreductible al 11925 C (0,40 %) a pesar de 5 tratamientos.

Por lo tanto, estamos en presencia de un quimioterápico que cumple con las premisas fundamentales del plan de la lucha contra la amibiasis:

1) Destruye la amiba en la luz y *pared intestinal:*

2) Modifica la flora favorecedora de las condiciones necesarias para hacer patógena la amiba.

Este paso adelante no significa que el 11925 «cura la amibiasis», pues ésta es un síndrome complejo que arranca con la instalación en el intestino de la amiba, pero no termina con la erradicación del parásito del mismo.

Este concepto debe suplantar a aquel, simplista, de que terminada la amiba, termina la enfermedad.

Creemos estar en presencia de un producto, y decimos así, pues seguimos estudiando su acción en una serie más amplia de enfermos, que nos permite no sólo abrir el primer frente contra la amibiasis, eliminando su agente etiológico, pues lo va a buscar en lo más recóndito de su ubicuidad, los tejidos, sino que comienza a modificar las condiciones locales del intestino *in situ* para evitar el anidamiento y los medios necesarios para su sobrevida.

Siempre que una amiba dysenteriae se instala en un colon hubo en éste una condición previa, de su continente y contenido que forjó el medio biológico necesario para que el protozoario «asiente reales» allí. Por lo que, en toda ocasión en que hemos tenido oportunidad, desde nuestro doble punto de mira de médico parasitólogo y médico laboratorista, hemos aconsejado estudiar no sólo la presencia ó ausencia del protozoario, sino también esas condiciones biológicas que permiten el anidamiento y parasitismo del protozoario (amiba dysenteriae) en el intestino.

## POSOLOGÍA

ADULTOS:

*1.º al 5.º día:* 1 gragea después del almuerzo.
*6.º al 15.º día:* 1 gragea después del almuerzo y cena.
*16.º al 20.º día:* 1 gragea después del desayuno, almuerzo e cena.

## CONCLUSIONES

1) Amibicida y bateriostático;

2) No se han registrado reacciones tóxicas en la sangre, riñon. Intolerancia sólo en 3 casos.

## BIBLIOGRAFÍA

Trabajos experimentales con 11925 y 11925 C en laboratorios Ciba-Basilea. 1954, 1955.

# A VERBATIM RECORD OF THE INFORMAL SESSION ON AMOEBIASIS

Dr. Brian Maegraith was in the Chair and 55 members of the Congress signed the register.

This is a verbatim record of the proceedings taken on a tape-recorder. The gaps have been filled; translation and minor editing have been done.

The Session was divided into periods:

TAXONOMY AND TERMINOLOGY.
VIRULENCE AND PATHOGENICITY.
EPIDEMIOLOGY.
DIAGNOSIS.
NEED FOR THERAPY.

## TAXONOMY AND TERMINOLOGY

BURROWS (Tuckahoe) — *(not recorded)* opened the discussion with a mention of the points of difference between *Entamoeba hartmanni* and the small forms of *Entamoeba histolytica*.

SWELLENGREBEL (Amsterdam) — Wished to make three points. The first was that he and his School had accepted *E. hartmanni* as a species for many years.

Dealing with *Pseudolimax,* he said:

*(Recording begins)*... out of a certain, as it later proved to be, misplaced generosity, we said if, for some reason or other, this genus

is not accepted as a separate genus, it may be dropped. Then, Dobell used this argument — he also recognised the necessity of a new genus, but he used his argument to drop our name and to create a new one which was ... No, well, I will not qualify that treatment he gave us. On the plea that *Pseudolimax* was not a good name because it was not a slug. Now *Pseudolimax* does not say that it is a slug — it simply means that it looks like a slug and he gave it another name which was *Iodamoeba*, which was just as bad because that amoeba was not a violet amoeba as the name implies; as if we don't take it so literally, which might imply that it is an amoeba which has something to do with iodine. That again is nothing special because it simply means that the cysts contain a glycogen vacuole, and any cysts of any amoeba, both dysentery and *coli* and *Endolimax nana* do — not *hartmanni*. That is the explanation of that word *Pseudolimax*. I am eternally grateful to Brooke, who was the only one in the whole world who did us justice. No one else ever did. That's my second point.

My third point is also a point of nomenclature. Kuenen and myself, in 1913, established the existence of two different stages of development of the dysentery amoeba, the *minuta* stage and the *histolytica* stage. Now the name «*histolytica* stage» has now been in existence from 1913 onwards, and you may compute for yourself how long that is. And now Hoare, all of a sudden, comes and calls it *magna*. Now I wonder why. Of course, this is not under the rules of zoological nomenclature, so everybody can do what he likes; but I think it really not very convenient to take away a very good name, because that stage is really a *histolytica* stage, and put in its place a name which doesn't tell you anything at all. That's not the essential thing.

So I must say, I must again protest against this change of nomenclature. That's all I wanted to say on this subject.

Kessel (Los Angeles) — The study of cytology of intestinal amoebae is most fascinating and stimulating. I had my early experience with Prof. Kofoid, who was an excellent cytologist and might be described as a «species splitter». For example, his species, «*lafleuri*», «*disimilis*», «*tenuis*». When out on my own, I considered that the morphological differences often graded into each other, and could not be confirmed by culture and other physiologic differences, so I

was content to designate the proposed species merely as strains, for example, *Entamoeba coli* one strain, *Entamoeba lafleuri,* another strain; *E. histolytica* one strain. *E. disimilis* another strain; or, I would say *disimilis* a strain of *histolytica; Endolimax nana, tenuis* a strain of *nana.*

Now the differences between *hartmanni* and small race *histolytica* described by Dr. Burrows are apparent to the microscopists. The first question is: Are they constant from culture to culture? Can you trace them through for a long period of time, and come out with the same thing you started with? The most apparent differences are the square-end chromatoidal bodies. Second, the eccentric blob on the periphery of the nucleus. Third, the lack of glycogen in iodine and the presence of your dispersed vacuoles in the haematoxylin stain. If these differences are constant, then one must be consistent and not draw the line at the 10 $\mu$ level. If desired, one would find just as many *histolytica* with crescentic bodies on the periphery of the nucleus in cysts above 10 $\mu$ as below. Now, what are we going to call these? Are we going to call them *hartmanni,* or are we going to extend the name *E. disimilis,* which was proposed by Kofoid around 1929, 1928? Now the difference between *hartmanni* and small race *histolytica* is really an academic one, if both are non-pathogenic. My main objection to the creation of this new genus is that it complicates the work and the difficulties of the clinical diagnostician because, if they are both non-pathogenic, why do we wish to make these differences?

Nelson (Virginia) — Those of us who have concentrated on cultivation procedures, primarily as a diagnostic tool, of course, but also in an effort to study the biology of *E. histolytica,* have, I think, all come to a conclusion — an agreement — that the very small *E. histolytica* — the one that certainly the evidence indicates should have a separate designation such as *E. hartmanni* — is different biologically from *E. histolytica* of either the pathogenic or the non-pathogenic type. This small amoeba is the one that gives you your failures in efforts to isolate the strain more than any others, more than larger strain, or the larger type.

Now, of course, when you are making cultures from stools for diagnostic purposes, you will have your positive cultures, and you will

have your negatives, and we find that most of the negatives — our greatest share of negatives — come with the small race, the small form. It is difficult to grow. Shaffer of Chicago has done a great deal, and made many efforts to isolate and grow this organism. It is only within recent years that he has had sucess, and that has been my experience as well. I think that closer study of this small form will reveal biological differences. There are evidences already in the observations we have made, both in its feeding activity, and possibly in the bacteria which it selects and is ingesting. I think that there are differences also in the feeding on starch. It is a small form, consequently it doesn't tend to engulf large particles of starch granules. Possibly this accounts for a lesser content of glycogen. I think that biologically there may come eventually evidences of difference between the form we will call *E. hartmanni* and *E. histolytica.*

Brooke (Atlanta) — I am not sure that the difficulty in the isolation of the small organisms is as justified as the precious speaker may have implied. We have for some time not been differentiating *hartmanni* at C.D.C., but we have been careful to separate those that are smaller than, say, 9 $\mu$ in the diameter of the cysts, and their trophs. from those that are larger. Miss Norman, in our cultural laboratory, has been quite successful in the isolation of the small organisms *E. histolytica* or whatever you wish to call them — by the addition of penicillin and streptomycin to the cultures. Then it is also necessary to examine the sediments more carefully. I think one of the reasons that we do not find them as frequently positive is that we do not stain the sediments as frequently as we should. By putting the sediments into PVA fixative, it is possible to make stained slides of culture preparations quite easily. Miss Norman has been successful in finding quite an increase in positives of small organisms by that method.

Beaver (Tulane) — I would like to suggest that, while we would, most of us I think, accept *hartmanni* as a species, we will have to do a better job than has yet been done in identifying a true small race *E. histolytica.* Generally, we identify our species as species and as colonies. The individual very small amoeba need not be identified either as *hartmanni* or as *histolytica,* but, if the whole population

of a stool is either large or small, there is no problem. Now, in all of the reports we see of surveys we get either *hartmanni* or some other name, without any problem. Many people report *E. histolytica,* small race, without any doubt. Now the fact that we can get small races of *E. histolytica* without doubt is the important thing. What we call it is less important. It is a separate species, I would like myself to call it *E. hartmanni,* and to abandon the term small race *E. histolytica* until someone, Dr. Burrows or someone else, can establish a true small race of *E. histolytica* — not a small individual of a population, but a small race of a species — small enough and stable enough that it can legitimately have a race designation.

Burrows (Tukahoe) — I agree with Dr. Swellengrebel that the original description by Prowazek left much to be desired, as were numerous other organisms which were described at about that same time. However, the description that he gave, later, of that species was much more accurate, but, at the same time, we cannot say that Prowazek did not see the same amoeba. Some day that question may be settled.

The next problem is the crescentic bodies that Dr. Kessel described as being present in the large amoeba. That does occur very frequently in the cyst. In the cyst of *hartmanni* the crescentic bodies are no more prominent than they are in *histolytica,* but it is in the trophozoite that those large bodies, sometimes crescentic, sometimes other shapes, enable one to distinguish that species from *E. histolytica.*

Now, as far as the appearance of this in culture over prolonged periods of time is concerned, Dr. Elsdon-Dew, who has done much more culture work, is better qualified to answer that phase than I am. However, from the standpoint of diagnosis, practically any amoeba which is under 10 $\mu$ will be *hartmanni,* because the small or the dwarf form of *E. histolytica* apparently is much less frequent. So I think, in general, that if we still use 10 $\mu$, those that are under that will in most cases be *hartmanni.* Dr. Elsdon-Dew has also found that the size of *histolytica* can be altered in culture. He can also explain that angle.

Schneider (Paris) — I say I am very surprised, personnally, to hear speaking of race when you speak of a large amoeba and a small

one. Why is it a race? When a man has been starving for a long time, he becomes thin; and when he has been well nourished afterwards, he gets the figure of a great, fat man. Has he changed race? Well, I believe, and I think things will prove what I say, that in a patient at one time with acute dysentery, you will find *E. histolytica* in the stools. In the same patient, coming back to Europe, treated or not treated, cured and improved, you will find in the stools some small forms, but I agree that they are the same. At one time they were nourished with good organisms and he became fat and active. At another time the intestinal microbes are not the same and perhaps he is not well fed and becomes small. This is a very simple idea, but I think it may also prove the other thing I wanted to say about isolation in cultures.

Some people say we can isolate very easily the large strain in stool culture. It would be much more difficult to isolate if it is a small race, but I think the reason is when you isolate the amoebae in the stools, you don't take amoebae alone, but you also take all the microbes and you give him his food to go on. This is the reason why cultures are easy. This is my point of view, and it is perhaps a very stupid one, but I felt I have to say it.

CHAIRMAN — Roughly why talk about races, because if you feed a nice fat *E. histolytica* badly it will become a nasty little, thin one and is still the same organism, but people will call it by a different race. He says that's his idea. It may be a stupid one, but he's sticking to it.

ELSDON-DEW (Durban) — This is a problem which has interested me considerably and I can see already that there is a certain amount of difficulty with nomenclature. To my mind, we are not always talking about the same thing. As I understand the position with *E. histolytica: E. histolytica* sensu stricto can be varied in size and will vary considerably from individual to individual, and you can even repeat this whole issue in a test-tube if you want to. By varying the flora with which you are growing *E. histolytica* you can either obtain large *E. histolytica* or small ones, but, over and above these, there is a small amoeba of the type described by Burrows which remains small, and it doesn't matter what you do with it, you can't make it big.

If you take a strain of *E. histolytica* sensu stricto shall we say isolated from a liver abscess, and grow it in mono-bacterial culture, or how you wish, it doesn't matter. Grow it together with an *E. histolytica* from an acute dysentery, put the two into the same culture tube, you will find that you have a single curve, if you will do the size-distribution. On the other hand, if ou take this little amoeba with the characteristics described by Burrows, do the same thing with it, you will not get a merging of the two populations. They will remain discrete in the same environment. In other words, I think they must be regarded as different species. But I would like to see some clarity in people's minds as to what they are talking about when they talk about large and small race because I do believe that *histolytica* as it appears in the stool will appear either large or small. I quite agree that the large ones are usually the pathogenic ones, and the smaller ones are the type you see in the carrier state, but there is another amoeba, and I think that is the amoeba to which Dr. Burrows is referring.

BEAVER (Tulane) — Before we leave this I would like to, once again, ask the question — if we do accept *hartmanni,* do we have anything left that we can call small race *histolytica?* We are not ready to leave this subject until we have met that question. I wouldn't want to propose that we try to settle it in a democratic way. Reminded of the Pogo Strip, when there is a question of what is a Gold Fish and what is a Gold Finch and it is settled by vote — incorrectly. We might do that here, but still there is a fundamental question that if we accept *hartmanni* — I do, many do — is there anything left that can be called «small race *histolytica*»?

CHAIRMAN — Dr. Beaver, may now ask you to take the vote?

BEAVER (Tulane) — Very much of a privilege to be able to count the hands. I count in favour of my views maybe. How many would recognise three *Entamoebae:* namely, *E. hartmanni, E. histolytica, E. histolytica* small race? How many recognise three amoebae? Those three I mention. Or, put it another way: Is there a small race *histolytica* that can be recognised separate from other amoebae? How many say yes?

CHAIRMAN — Cheating going on down there.

BEAVER (Tulane) — I counted hands. How many are there among us who would say, «if we recognise *hartmanni,* there is then remaining no such thing as small race *histolytica,* as a separate species that can be recognised in stool, can be propagated, and has taxonomic status»? How many say yes? Now be careful.

*(Background voices.)*

You would claim that you could go into a population, examine stools and record your findings. We would do stools on everybody present, and we woul find *E. histolytica.* We would find *E. hartmanni.* Would we find small race *E. histolytica* also? How many say we would? We would find small race *E. histolytica* also — as a separate species — as a separate entity?

*(Background voices.)*

Well now, I shall have to start all over. Shall I begin again, Sir? The zoologist: let's forget for the moment that we are interested in the patient, and that there are pathogenic and non-pathogenic; we forget that now. As from the zoological point of view, there is such a thing as a species. Do we all agree? There is a species. There also, among species, are variants and these variants are legitimate or not, only if they are stable. Now, *E. histolytica,* like other species, may occur in colony form, somewhat larger or somewhat smaller — we agree. Now, there is also *hartmanni;* presumably it would also be large or small. Dr. Burrows and others would regard *hartmanni* as not getting larger than 10 $\mu$ — not getting larger as a population than 10 $\mu$. *E. histolytica,* we are told, varies all the way from very large amoebae as population, to small amoebae as population, so that we might expect in a patient, or in a test-tube, *E. histolytica* as a population less than 10 $\mu$. My question is centred on that statement — that *E. histolytica* may exist in the bowel or in the test-tube, as a population, less than 10 $\mu$ and therefore confused with *E. hartmanni.* Do I make myself clear now?

Right, how many would agree that we are likely to find in any population of people amoebae in the stool, less than 10 $\mu$, and properly called *E. histolytica?* Those who would recognise *E. histolytica* in the strict sense, truly *E. histolytica,* less than 10 $\mu$ and different from *E. hartmanni.*

SWELLENGREBEL (Amsterdam) — I do not agree with you that that would be a hereditary character.

CHAIRMAN — That point is taken.

BEAVER (Tulane) — It is not a hereditary character. Well, you see I find that we cannot, even at this time, put a question clearly, let alone get an answer to it.

*(Background voices.)*

The opposition. How many recognise *E. histolytica* on the one hand, *E. hartmanni* on the other hand and do not have trouble with a small race? How many of us are in that position? A small race of *E. hystolytica?* How many among us would not find it necessary in doing a survey to even mention small race *E. histolytica?* If we do a survey of everybody in Lisbon, would we report *E. histolytica, E. hartmanni, E. coli, Iodamoeba* (*Pseudolimax,* that is) and so on, without mentioning small race *E. histolytica?* How many would find it unnecessary to mention small race *histolytica?*

*(Background voices.)*

If Prof. Swellengrebel were surveying the population here, you would not mention small race. How many others would not mention small race? Prof. Neghme would not. Prof. Woodruff would not. I see many others would not. Prof. Kessel, you would — between small race *histolytica* and *hartmanni?*

*(Background voices.)*

I was going to ask Prof. Kessel if he would tell us on what basis he would recognise the small race now.

KESSEL (Los Angeles) — On a basis of the morphologic differences given by Dr. Burrows, *E. histolytica* has, in both the trophozoite and encysted form, nuclei with central karyosomes predominating, with evenly encrusted chromatin on the periphery of the membrane. The cysts have chromatoidal bodies which are rounded. In contrast, the cysts of *hartmanni,* as described by Dr. Burrows, have chromatoidal bodies which have predominantly square ends. That's troubled us for a long time, but that differentiation is very helpful. Likewise, it has scattered vacuolation in the encysted (I believe) stage. Correct? While *histolytica* tends to have a large single glycogen, now *hartmanni* cysts do not show this to be glycogen, but in the haematoxylin-stained slides you can see this difference.

So I would make my difference primarily on nuclear structure, chromatoidal body-shape and structure of vacuolation in the stained cyst.

SWELLENGREBEL (Amsterdam) — May I ask one question? This Session is divided into sections. One is called «virulence and pathogenicity» and the other is «epidemiology». What I have to say on the subject, I am not quite sure whether I ought to put it in «virulence and pathogenicity» or in «epidemiology». I shall ask you to give me the floor under «epidemiology», but I am almost sure you will tell me then, «Oh, but that belongs to virulence and pathogenicity».

CHAIRMAN — I'll see that I don't miss that point.

## VIRULENCE AND PATHOGENICITY

KESSEL (Los Angeles) — The question of the differences between virulent and avirulent strain of *histolytica* has interested those of us who worked with amoebiasis for many years. I did some work in the earlier part of my career and, subsequently, Dr. Meleney and Dr. Frye did a masterful piece of work, in which they established the pathogenic index to evaluate the virulence, shall we say, of these strains in kittens.

We call *Corynebacterium diphteriae* a virulent strain and an avirulent strain — we don't give them different names. So, they

retained *E. histolytica,* and had varying virulence. Now, in one of their studies, they found that most strains from acute cases had a high pathogenic index — of 3 or above. However, they also found that strains from carriers had a similar high pathogenic index occasionally.

Now Dr. Neal's report the other day was of extreme interest, and his chart showing *E. histolytica* from carriers more os less corroborated this finding, although his work was done in rats. There was at least one strain that had a pathogenicity equal to the virulent strains from acute cases which he reported.

Now this being the case, I think we are rushing things a little bit too much to disregard the fact that there may be carriers with virulent strains. I think Dr. Neal has proved that as well as the previous workers. I have heard several times at this conference that virulence is a variable factor, depending on environment on which the amoeba is growing. Until an easy way is found to differentiate strains from highly pathogenic and from non-pathogenic but with a high pathogenic index and with a low pathogenic index from carriers, I think we are justified in retaining our old practice of treating people who harbour *histolytica* — like *Entamoebae.*

Neal (London) — I am well aware that the work I have done is not the complete answer to the question of pathogenicity. There are many things of interest left to be done. For instance, these cases which have no symptoms, but in which liver abscesses form. Presumably liver abscess means that amoebae got into the tissue and then into the liver. It would be very interesting to do some experimental work with strains from this type of people. Another thing of interest is that we know nothing at all about the effect of encystation on transmission of virulence and, in that way, the possession of virulent strains in so-called carriers comes into the picture. We don't know whether the virulence is transmitted by a cyst, or whether encystation automatically means a loss of virulence.

There is another thing in the definition of the word *strain.* I have to point out that I have used a very narrow restricted use of the word.

*(Background voices.)*

I have used that an isolation of an amoeba from one person constitutes one strain; and the isolation of the amoeba from the same person a month later I have called a second strain. Now the reason for that is that *in vitro,* although the amoebae are presumably the same, it has a different bacterial flora which gives it different growth characteristics.

WOODRUFF (London) — On this question of virulence, Mr. Chairman, I think there can be little doubt that the *hartmanni* strain is avirulent as compared with the larger strain. We have been interested in this problem in my Department in London for the last two and a half years. Dr. Curzene has there been working on it particularly. Using the criterion of the size of the cysts, we have tackled this problem from three angles. Cysts have been measured in individuals, patients attending at the hospital, over a prolonged period. We have been able to get specimens from untreated patients, in some instances for up to 9 months, and we have attempted to see whether the various races breed true in the individual and, in brief, they appear to do so. Both the large and the small appear to remain at approximately the same diameter throughout these periods in single individuals. The large doesn't change into the small, nor does the small change into the large.

And then, secondly, she has attempted to culture small and large race and, as other speakers have made it clear, the percentage of successes in the small is much less than in the case of the large. Nevertheless, she has isolated some seven or eight strains of small race in culture and has been able to put these into weanling rats and, using the technique of Jones, there has been a clear difference in the degree of damage done to the intestine of the rats by the small, as compared with the large. A statistically significant — quite clear — difference in the infectivity to rats.

Thirdly, we have been interested in correlating the symptomatology of those who exhibited the small organisms in the stool with those who have the large, the *hartmanni* and the large. We found that there was a significant correlation between symptoms in patients

who passed *E. histolytica* and there was no such significant correlation in those who passed the small *hartmanni*.

The small *histolytica* I personally would regard as a particular phase, a resting phase, of the large *E. histolytica* and I think that is the view of Hoare and that if you measure the diameter of cysts and use the criterion of 10 $\mu$ to differentiate between *E. histolytica* and *E. hartmanni*, then you probably won't go far wrong.

*(Background voices.)*

No, as a species, that is why I especially asked Dr. Beaver if he meant as a species that propagates itself. I especially asked him that very point.

*(Background voices.)*

NEGHME (Chile) — We have had the opportunity in our country to follow the evolution of the clinical aspects of amoebiasis for over forty years, that is to say, from 1912, when emetine was introduced in practice for the treatment of the patient, up till now. We have observed a change in the virulence and the pathogenicity of the *E. histolytica* strain of our country. Because in 1912 it was very common to see many acute amoebic cases, liver abscesses occurred rather frequently. We have records that, at that time, in the wards of Clinical Hospital, Santiago, at least one or two patients presented with amoebic liver abscess and acute amoebic dysentery was rather common also. After that, these cases, every year once emetine was introduced, became less and less frequent and now it is very unusual to see patients with liver abscess or with acute amoebic dysentery. Most of our infections with *E. histolytica* are rather chronic or asymptomatic individuals. That is to say, we have infections with *E. histolytica* and in only some cases the disease — the chronic disease rather than the acute disease. Dysentery is rather infrequent.

Studying this from the parasitological point of view, we have found sizes from 5 $\mu$ to 11 $\mu$ in patients coming to the Out-Patients Clinic with chronic intestinal disturbance. Most of our cysts were from 7 $\mu$ to 8 $\mu$, and only 0.4 were from 11 $\mu$. If we take 10 $\mu$ as a mean measure of the cysts to differentiate *E. histolytica* as small race

from the larger race, then, it seems to me, we practically will not have *E. histolytica* at all in Chile, in spite of the fact that we have many patients with dysentery and with symptoms of amoebiasis in general.

STAMM (Royal Air Force) — I didn't accept your invitation before because I was rather in the same position as Dr. Swellengrebel as to whether this was going to be epidemiology or pathogenicity. Dr. Hoare's papers have suggested recently that there should be two separate races of the large race of *E. histolytica,* one pathogenic, confined to the tropics, and one non-pathogenic, cosmopolitan, and Dr. Neal also suggested the other day that, in his opinion, there was a possibility that the non-pathogenic amoebae in his laboratory (he was unable to make them pathogenic) and he thought that the pathogenic amoebae might derive from the non-pathogenic at certain times — I feel rather that the non-pathogenic was derived from the pathogenic.

I have a certain amount of evidence that I think bears on these points. We had some Pakistanis, apprentices in England for three years, 181 of them, and we examined them, three examinations each year, for three years. During that time we picked up on the 9 examinations 101 with *Entamoeba histolytica* in their stools. Of those, only one ever suffered from any symptoms attributable to amoebiasis. During that time they maintained exactly the same rate of carrier state, so that here were 181 Pakistanis straight from Pakistan and yet apparently in England the race was non-pathogenic.

I feel that it is quite unnecessary to suppose that there are two distinct races of the large strain, one pathogenic and one non-pathogenic. I think it is a question of environment and, from our experience of other organisms, it is far more likely that some factor like rapid passage under insanitary conditions enhances pathogenicity, rather than round the other way. When they come to a place where sanitation is good, what one is merely doing is maintaining an *in vitro* culture in the lumen of their gut. We know that a pathogenic strain kept in an *in vitro* culture for a long time loses its pathogenicity, and that is what is happening, I think, in their gut.

NAUENBERG (New York) — I wanted to say during our work in Colombia, South America, we have observed that among children that

harbour the amoebas and that have no symptoms during a long time, as soon as they got the measles, invariably they would get amoebic dysentery afterwards as a complication as frequent as bronchitis or otitis and that, during the rainy seasons, the amoebic dysentery would be much more frequent than during the dry season.

NEGHME (Chile) — My idea was that the introduction of emetine made tremendous selective pressure on the population of amoeba in the world and so the most pathogenic strains have become whittled out, and the strains which we have now are less pathogenic than they were forty-five years ago because of this.

BROOKE (Atlanta) — Perhaps this would be a good occasion to get some help with some interpretation of the data which we collected in South Bend, Indiana. In that investigation, we found 800 individuals infected with *E. histolytica*. We were not in a position always to study them carefully, so we do not know what percentage might have been small race or *hartmanni* or what. In this investigation the epidemiologists and nurses took the G.I. history on the 1,600 individuals in this plant prior to the stool examination. Four stool specimens were examined on each patient or individual.

After the investigation was over, we started tabulating the symptomatology of the people which were infected and not found to be infected. With the exception of weight loss, we found more symptomatology in the negative individuals than in the positive individuals. This was in a situation, however, where there had been at least 31 recognised cases of amoebiasis. There had been four deaths, at least two or three of these had demonstrable liver abscesses. So if you have some explanation for this, we would be most appreciative to get it. It looked almost like there is an all-or-none situation.

CHAIRMAN: What you are really asking is for someone to come forward and try and explain exactly what he means by pathogenic. While he is also doing that, he might also describe virulence. I think it is a great pity that we haven't had more talk about the relation of the host to the parasite.

*(Background voices.)*

JACOBS (National Institutes of Health) — I was very interested in Dr. Neal's statement that he designates a strain as an amoeba of the origin of a particular patient and another strain is from another patient. As a matter of fact, this is about all that we can definitively say about strains of *E. histolytica,* at present. It's a designation of convenience, for our use in the laboratory, because we have not established solid criteria on which to base the definition of strains.

The question as to whether or not strains are mutable has also not been answered. We can make certain analogies with other protozoan organisms which are mutable, which have been changed under various circumstances. We don't have to stop with protozoan organisms either; in the entire context of microbiology, we run across this situation of different strains all the time. Certainly, we can suppose, at present, that there are different strains of *E. histolytica,* but that is all we can do because we cannot define them. I would suggest that if strains are to be defined, they be characterised on the basis of their invasiveness and pathogenicity when they are cultivated for precise periods of time with particular microorganisms, or, if the ideal is ever reached, all by themselves. I think, at the moment, that is about all we can say about strains. Anything about the susceptibility of the host, of course, will also be conjecture, until we can know precisely what the pathogenicity of the particular organism is that we are dealing with at any particular time.

DE CARNERI (Milan) — *(translation from tape)* It would be interesting to know whether the strains of amoebae isolated from the Pakistanis by Stamm, which had become avirulent by prolonged existence in England, merely had been debased or whether they have really completely lost their pathogenicity.

We know that in culture prolonged over many years, pathogenic strains of *E. histolytica* slowly lose their virulence, but one can restore this virulence by injection into the liver of hamsters or by other methods.

Perhaps, as for other protozoa, for example it has been demonstrated for certain trypanosomes that, after years of cultivation *in vitro* in saprophytic conditions, *Entamoeba histolytica* may lose forever the

capacity to secrete the enzymes which enable them to penetrate the intestinal wall. I have cultivated, over many years, strains isolated fifteen years ago, thirteen years, eight years and three or four years, which had been pathogenic for man and have attempted to find out whether the virulence lost by prolonged cultivation could be reactivated by passage through hamster liver and found that old strains regained less virulence than those recently isolated.

That is to say, for example, a strain originally isolated by Dobell is now completely avirulent, but if it is passed through a hamster liver accompanied by virulent bacteria, it can ulcerate 11.3 % of injected rats. Other more recently isolated strains which had also completely lost their virulence *in vitro* can, after passage through the liver, ulcerate 100 % of the rats.

It would be interesting to deduce a possible evolution and the establishment of a completely non-pathogenic race which lives happily as a saprophyte, a phenomenon which has occurred in the evolution of many parasites in losing their pathogenicity. It is well known that more virulent strains have less chance of perpetuating themselves because they do not regularly form cysts.

CHAIRMAN — I think it roughly concerns the business of the loss of virulence in long culture whether the virulence is attached permanently to the race or strain in particular or not.

*(Background voices.)*

STAMM (Royal Air Force) — If I understand that correctly, it seems to me that it answers a point which I have always thought was the correct answer to the inability to make non-pathogenic *E. histolytica* become pathogenic in the laboratory. Many people, on the basis of that, have said, Hoare for one, have based the idea that there are two races but, if our French colleague has managed to make them pathogenic again by passage through hamsters, then it confirms my previous suspicion that it wasn't the *E. histolytica* that was wrong, it was merely our laboratory technique; and that all we need to do is to find the right technique and I am quite sure that they'll become pathogenic again.

HARINASUTA (Bangkok) — *(edited version):* I have heard of the determination of the virulence and pathogenicity of *E. histolytica* by the use of animals. Is there anyone here using an *in vitro* method? Four years ago, I developed such method to which but little attention has been paid. I repeated this work on my return to Liverpool. The method is easy and quick and consists in the application of washed amoebae to an exposed and developed gelatin film. Unhealthy amoebae have no effect, but on a good medium they digest very well and probably have proteolytic enzymes. The culture was old and had apparently lost its virulence, but, after repeated animal passage, the gelatin film was once again well digested. Probably the proteolytic enzyme has something to do with virulence. That is the first experiment.

The second method I used was digestion of a tissue. I put amoebae into a tissue (I have my special technique) and the tissue was digested as determined by the amino-acids shown on paper chromatography. This we will publish later.

MAEGRAITH (Liverpool) — The idea that the *Entamoeba* has a gelatinase I think is fairly firmly established. What isn't established is the relation between the presence of that gelatinase and any pathogenicity that the organism may possess. So we decided that it might be better to try to find out whether the particular organism, with which we were working, would in fact attack epithelium itself. To do that we obtained a fresh human large gut, dissected off the epithelium by quite a simple technique and measured the amino-acid content of the fluid in which the epithelium was suspended at a fixed time after adding *E. histolytica*. By identifying the amino-acids, we were able to show quite conclusively that this particular organism did in fact digest epithelium. It has always been said that it could digest epithelium.

There has been one or two rather inconclusive experiments trying to prove that point, but I think that Dr. Harinasuta is now demonstrating this for the first time, that this is quite definitely a proteolytic enzyme which can attack the protein of epithelium. How closely that is related to pathogenicity still has to wait an answer until someone defines what pathogenicity is. I feel that pathogenicity has as much to do with the host, and the particular state of affairs at the time at

which the parasite meets the epithelium of the gut, as it has to do with the parasite.

## EPIDEMIOLOGY

SWELLENGREBEL (Amsterdam) — Mr. President, *E. histolytica* is a commensal. It has not a commensal stage, it *is* a commensal. What is a commensal? A commensal is a parasite, which, as a rule, keeps well within bounds, is subjected to what is called topical restraint by the host. That is to say, it keeps in the cavity of the nose, or the mouth, or the intestine, or the female organs. There are many commensals. There is, for instance, the meningococcus; there is the virus of poliomyelitis, which does also keep within bounds, but not in a cavity, but in certain tissue of the intestinal wall. The only difference between *E. histolytica* and other commensals, of which there are hundreds, which all pose exactly the same problem which we have been discussing now. Why is it not invasive at this time and why does it invade the human body or the animal body at another time?

There is nothing special about it. The only speciality of *E. histolytica* is that when it becomes invasive, it also changes its form. So long as it keeps within bounds — so long as it does not violate topical restraint, it is the *minuta* form. When it does violate topical restraint, when it invades the tissues, first of them, the gut wall, maybe others, then it takes up the *histolytica* form (I'm still using the old terminology of Kuenen and Swellengrebel of 1930), and now the point that interests us is why does it invade the tissues, and why do other intestinal amoebae not do so? We are never sure that others can't do so. There is not a single commensal which is really harmless — they always try to get in, but some succeed more easily than others. Even *Pseudolimax,* even the iodine cysts, have been known of once by Derrick to invade the tissues, not only of the gut, but of the whole body. But that was only one — that was an exception, but still it makes us understand that every commensal, including all the parasitic amoebae of man in the intestine, may at some time become pathogenic, that is to say (don't let me use the word *pathogenic* because our President strongly objects to that and he is right, but let's call it invasive),

getting into the tissues, and it is *histolytica* which does it more often than others, but any might do it as iodine cysts have shown.

Now the point is, why does it invade the human body? There are, I think, various reasons. It may be due to the parasite, it may be due to the host. Let's take the host first. The intestine of the host may be under stress, either by an over-rich and varied intestinal flora, as may be present, especially in hot countries. I particularly do not say tropical countries, but hot countries, following the French terminology of *pays chauds,* because a hot country need not lie in the tropics at all. For instance, Persia is a hot country, although the winters are very cold. Many parts of the United States belong to the hot countries, although the winters are very cold. So the simple effect of the heat may itself influence the intestine, but there are various other conditions, which are especially present in hot countries, which put a strain on the intestine of the host. That is because it is especially in hot countries that we find these so-called underdeveloped countries which are characterised, among other things, by the fact that faecal matter is very, very easily transported from one man to another, and very quickly so. That there is, to use an expression which has been used by my colleague Schneider, «a close faecal contact», which means a quick transport of small particles of faecal matter from one person to another. In this way there is a quick transport of bacteria and we know that frequent passage by itself may enhance virulence. The same applies to *E. histolytica* in the body of the various persons in these underdeveloped countries. In, let us say, Holland, where 10 % of the population are infected with *E. histolytica,* but they simply remain in their bodies. There are very few transports from one to another. There is a certain amount of transport; it can't be otherwise or the whole of the species would die out, but it's not so frequent as in the underdeveloped countries and, I repeat, underdeveloped countries are very often found in hot countries, especially in the tropics.

Then there is another thing which is of importance in underdeveloped countries. In underdeveloped countries there is very often food deficiency and there are various authors, and our Secretary (are you the Secretary?) among them (well, really the author intellectual of this whole meeting), who have shown that protein deficiency induces and stimulates the activity of the amoebae. So all these, I can

only touch upon it very briefly, refer to the host, and not at all to the parasite.

But now we come to the parasite. The parasite may be induced to more activity, that is more invasive activity in two ways. It may be that simply by frequent passages virulence is enhanced, but it is also possible, and I do not want to deny that in the least, that there really are races — hereditary races — of *E. histolytica:* some of them less prone to invasiveness, others more prone to invasiveness. If they exist, I don't think they are restricted to two races, one who never invades and the other who always invades. No, there might be a whole range, gamut, of more or less proneness to invasiveness.

This is to my mind really the story of the epidemiology of amoebic dysentery.

Elsdon-Dew (Durban) — I think I am in a particularly favourable position to study the epidemiology of amoebiasis, but, even though I am in such a favourable position and have been working on it for ten years, I am still not clear in my own mind. As you probably know from my writings on this subject, we are confronted by a particularly virulent form of amoebiasis which occurs in our native African population. It is not in any way parallelled by a similar disease in the White population or, for that matter, in the Indian population.

Now that there must be interchange of parasites between these various three peoples is fairly obvious, because the White people, for example, have Africans as their cooks, and so on, and so the faecal interchange which Dr. Swellengrebel has mentioned must be taking place pretty well all the time. Yet, in the African, we have that acute picture which was demonstrated by Dr. Roach, and in the White people to all intents and purposes that does not occur.

Now what are the underlying features? There is no doubt that the African, in general, is living under much worse conditions of hygiene, but the wild man, that is to say, the African out in the bush, doesn't get it. It is the peri-urban African, the African who is working in the towns, who gets it. Another complicating factor is that in Lourenço Marques — a city about 300 miles north and therefore more tropical than we are — the Africans don't get it.

A very high proportion of our cases come from a restricted section of our population. These are people who are daily labourers, that is to say, work by the day. They are separated from their families and they are, generally speaking, living in all kinds of places, some of them under the worst possible slum conditions, but, equally well, others again are living in barracks with adequate hygiene.

These are the people who supply the great majority of cases and there is only one thing which seems to be rather characteristic of these people and that is their diet. They are, as a rule, young men, or youngish men anyway, coming to the great city to seek their fortune. They expect the streets to be paved with gold and, in point of fact, they are actually handling more money than they ever did in their lives before in the bush. Their tendency then is to copy the White diet and the way they copy the White diet is to exist on what we have come to know as «the bun and lemonade diet».

Now, that is merely an epidemiological observation. The other point where we have a certain amount of evidence, I think, is the remarkable success we have had in using antibiotic therapy; that, I think, indicates that in some way the bacteria must be playing some part. I think that the rapid interchange of faeces between one person and another undoubtedly plays a part over and above the question of the transmission of the various protozoa. It may also mean a rapid interchange of bacterial flora amongst these people. To me the position is still a problem, and I had hoped to get something from somebody else to give me some more ideas.

BERBERIAN (New York) — In 1948 I carried out a stool survey in the Albany, New York area, examining the stools from about 500 persons. On the average 1.6 stool specimens per person. As a result of this examination, I found that 8 % of the people had *E. histolytica* in their stools. I lived in that area nearly 11 years and I have been carrying on studies of stool examination on the people, regardless of their symptomatology.

I have observed a definite trend — the percentage of positives has been coming down year by year and I am sure that if I carried out a similar survey in 1958 in the same area, the percentage of *E. histolytica* carriers would not be more than 2 or 3 % at the most. I believe the principal fact in this reduction of carriers of *Entamoeba*

*histolytica* is the extensive use of wide-spectrum antibiotics. They merely are prescribed for various other conditions, and yet, at the same time, they eliminate *E. histolytica* infection in the intestinal tract.

BEAVER (Tulane) — I do not wish to belabour too far my original point, but I sense that we are inclined to bog down somewhat in our discussion of pathogenicity and epidemiology, because we cannot really agree on what it is that we deal with.

Now, Dr. Neghme's figures are very disturbing to me. He tells us that he finds in the general population an average sized amoeba that is too small to be called *histolytica*. Now we should like to learn from Dr. Neghme facts about the epidemiology and facts about the pathogenicity of *Entamoeba histolytica* in his country. We should, at the same time, like to learn from Dr. Elsdon-Dew as much as we can about *E. histolytica* in Durban. But now, unless we can be agreed upon what it is that we are dealing with, we shall then only be more confused rather than less.

Dr. Burrows has done us a very great service to return our attention to other features of the amoebae than its size. Surely, out of this meeting, there should come some kind of resolution that all of us will concentrate our efforts and attention to the identification of the «thing» with which we work. The results of our observations may then become interpretable. It is very disturbing to me that we could not, in some way, get some agreement on at least what direction we should take in the interpretation of data already recorded. That we do not have any place in the literature, amoebae identified into three kinds of *histolytica,* one of them being *hartmanni,* one of them being small race *histolytica,* one of them being large race *histolytica*. What will we do when we come to interpret data already recorded?

It is perhaps a good place to offer the suggestion that we try to agree, now, on whether we can in the future deal with *E. histolytica* as a species, that we can all interpret similarly with reference to its identification, its pathogenicity and its epidemiology. It would perhaps be helpful to hear from Dr. Elsdon-Dew what interpretation he is placing presently on the smallest amoeba he finds — call it *hartmanni* if he will — and the amoebae that he sees in an individual patient, who comes to him now with dysentery — he is a sick man — he gets treatment — the treatment is effective against his sickness,

but it does not remove the amoeba and the amoeba is now a *histolytica* of one kind or another. It is in this patient who is now well, and it falls into the category which he publishes — «the healthy carrier»: a sick man yesterday, now a healthy carrier. What kind of amoeba do you find in him, Dr. Elsdon-Dew?

Elsdon-Dew (Durban) — I think the kind of case to which Dr. Beaver is referring is this. In the course of our studies of the various antibiotics and other types of therapy in these acute cases of ours, certain types of therapy do not remove the amoebae, but we will find, after the patient has been cured of his illness, as Dr. Beaver says, that this patient is now passing cysts, whereas previously in the acute phase we never say the cysts at all.

Now are those cysts direct descendants of our acute amoebae, or shall we say our actively invasive amoebae, or are they descendants of some lumen dweller who has survived.

One of the questions which I cannot answer — I don't know whether anybody can — is as to whether the amoebae of the invasive type do naturally produce cysts. We have been trying to do this, and have had some slight success. because at one time I believed and I still have a sneaking desire to continue believing it, that is, that the really invasive amoeba is committing racial suicide. That is to say, that it will not have any chance of posterity.

Now, that means that the disease follows on some change in the amoebae from the avirulent or carrier state to the invasive form — a change which is apparently occurring in some part of our population, and not in another. That I cannot explain. I think that answers what Dr. Beaver has to say. I am still completely in the dark. I don't know!

Neghme (Chile) — Most of the cases we have seen in Chile for a number of years, present mainly with chronic diarrhoea, intestinal disturbance, dyspeptic troubles and sometimes constipation. Acute amoebic cases are rather rare, as are amoebic hepatic abscess. We have seen two or three cases of hepatic abscess in our area, and we found in them trophozoites of the larger race — the so-called large race — and we have several strains in culture in our laboratory from these acute cases. Also, we have been able to isolate amoebae from chronic cases, but we do have also a large number of individuals

infected without any symptomatology at all. These are the so-called carriers.

We have performed a general survey in the country, examining nearly 20,000 people from the northern to the southern part, covering all the country practically. We have found figures from 18 % of the parasite we call *E. histolytica* to 30 or 40 %, in some areas, of the same parasite.

Most of these infected cases are carriers, but clinicians feel that amoeba is producing chronic disturbance, and they treat them with antibiotic therapy or emetine, whatever the case is. They treat them, and in 25 % of the cases they obtain very good clinical results, but unfortunately sometimes the infection persists, and in those cases we find most frequently this small size of cysts of *E. histolitica*. This seems to me is in favour of the idea that we are dealing with two different species. In some cases we have *Entamoeba histolytica* and in the others we may have *E. hartmanni*.

Unfortunately we haven't paid attention to this *E. hartmanni* until now.

Otto (Chicago) — Before Dr. Beaver tries to settle another controversial and ambiguous question by the democratic process, I would like to make one comment on one aspect of the epidemiology.

Many of you are aware, I am sure, that in such things as amoebiasis of snakes, it is uncommon in the wilds but produces disease when you get them concentrated in a zoo. One sees the same thing with coccidiosis of poultry and quails, and other organisms as well. I had an opportunity, three years ago, to see what was almost an experimental set-up with children, in a similar manner.

This was a home for feeble-minded children, ranging in age from 1 to 5 years for the most part. They ranged all the way from morons at the upper extreme down to microcephalics and completely acephalics at the other extreme. They were divided basically into two groups. One group was bed-fast — about 150 were bed-fast, due to physical and mental deficiencies and not due to any infectious disease. The other group were semi-ambulant, crawlers, toddlers, etc., and in this latter group human faeces were scattered over the clothing, on the floor, in a room about this size.

And in these 120 children, everyone of them, at one time or another, showed infection. At any one point, about 25 % were acutely ill with amoebiasis. They occupied beds in the same room with the bed-fast patients. In the bed-fast patients there was not a single case of acute amoebiasis, and yet they were in reasonably close proximity. In so far as the organisms were identified, they were all large race, and most of the toddler group — so-called toddler group, and crawlers, etc., were passing trophozoites, but an occasional cyst was seen. I think this has important bearing on the whole question.

The intensity of exposure is extremely important when you come to the question of epidemiology of acute amoebiasis and probably has a differential relationship on the transmission at a lower level with production of less disease. I'm not trying to imply in this that I do not believe that there are different strains, races or species or what-have-you. That is undoubtedly true, but the question of intensity of infection is extremely important. In this situation I mentioned there were many complexities. In addition to the question of the intensity of the exposure on one hand and the rather nominal amount of exposure on the other hand. There are many other questions involved, but, to me, it is extremely important to keep in mind this matter of intensity of exposure as one of the factors.

THOMPSON (Detroit) — It seems to me that there are at least two determinants of amoebiasis, or amoebic infection, which we should certainly keep clearly in mind.

I want first to very strongly endorse the point made by Dr. Elsdon-Dew on the effect of diet. In every situation in animals where diet has been studied carefully there has been shown to be a distinct influence. This is where the strain of amoebae and the infecting circumstances, kind of animal, was kept constant there is no question that diet enters in every case has been studied.

The second point to mention is that there is no question that strains of amoebae do vary in virulence, but the analysis of virulence is a very treacherous affair. I have had the good fortune, or possibly the misfortune, of running many hundreds of experiments with the same strain of amoebae, with the same animal, and under the same conditions, from week to week, and we occasionally have complete failures in our experiments. We can predict these when we examine

the culture. We have no trouble in harvesting enough amoebae by exhausting all that the tubes contain and they look all right, but we know that the growth has not been as good as normal and those amoebae are not as able to develop either in the intestine of the rat or in the liver of the hamster, as are rapidly multiplying amoebae.

Brooke (Atlanta) — Several years ago you may recall that Tobie and his co-workers at Wassaic Mental Institution, New York, had excellent results with the use of terramycin in the treatment of patients there in the Mental Institution. For a period of 6 months, I believe, they found no one infected after the original examinations. We had an opportunity to do a similar study in another Mental Institution in Woodward, Iowa, and in that study, whereas terramycin was used, we were only successful in knocking it down to a rather low level, and over a period of six months it rose again to about one half of the original prevalence rate.

I think that this perhaps can be explained on the basis of Dr. Otto's comments in regard to the prevalence and the rapid spread of faecal material. In the Wassaic Institution, on the basis of about four examinations, they found a total of about 45 % prevalence rate, whereas in Woodward, on one examination, we found 65 % of the people infected. Now, in addition to it being a higher prevalence, I wish someone were in a position to give us some technique of perhaps determining intensity of infection. Maybe that would help us in evaluating the differences in these epidemic situations.

We've been talking a great deal about the pathogenicity, when we should be talking about epidemiology at this particular time. I think in the United States of America, where we have a great deal of chlorinated water, we may be living in a situation where we are under false sense of security, because of the common method by which we determine bacterial contamination of water supplies using bacterial methods. I wish there were some really effective means of determining sewerage pollution of water supplies so that we could really test the water in the various situations. In the United States many of the plumbing supplies were put in 40 to 50 years ago, and there have been many alterations and if we had some method of perhaps determining local contamination within a building, it would help us perhaps to avoid the situation that occurred in South Bend.

STAMM (Royal Air Force) — There's a point that Dr. Brooke made just now, I think just one comment perhaps worth making is on the question of water supplies. I don't think sufficient attention has been given to this matter in the past; on water as being very commonly, probably, the origin of the amoebae. I was very impressed, when I visited Malaya, to find that the troops who were on jungle warfare had a far higher amoebic rate than any of the others. In fact, the others had practically nil. I enquired into this, and found that all their food was supplied by air in tin containers and so on, and the only thing that they took in during their jungle warfare was water, which was taken from local streams and chlorinated in their water bottles. So I then had a number of samples of the chlorine tablets issued from the quartermasters' stores and had them analysed. 75 % of them were deteriorated to below a quarter of their supposed content. I have very little doubt that that's where they were getting their amoebae.

SWELLENGREBEL (Amsterdam) — Following on what was said just now, that there's too little attention being paid to infection by drinking water, I may remind you, though most of you have never heard of it, that in Sumatra, among the workers on the plantations of tobacco and rubber, amoebic dysentery was of great importance in the early days of the present century. Schuffner and his colleague Kuenen applied the following method. Of course, these workers were working in the field, and what is easier than to drink water from the clear brooks coming down from the hills, what clear running water but especially dangerous in this respect because in this clear, aerated water that the cysts may survive for a long time, as Kuenen and myself have demonstrated later on. These were the years 1903, 1904 and so on.

And then we instituted the following measure. Tanks, filled with hot tea, which gradually cooled of course, were driven in the fields. They were so small that they could easily roll along even on small carts and the workers in the field filled their canteens with this hot tea. That was what they were driking. That had a remarkable success. Of course it's always very difficult to know exactly what is the effect of any measure, but it is certain that after these measures have been introduced, amoebic dysentery didn't disappear, but it went down to such an extent that it was no longer a major problem. Now this

is a very old story of more than 50 years ago, just to show you that certainly much attention had been paid to water as a medium to transmit amoebic dysentery.

MORETTI (Bordeaux) — *(translation from tape)* I feel that our experience in Morocco corroborates the view of the previous speaker.

The disease was extremely prevalent in Morocco, particularly in the region of Fez, where the water was, at the time of the arrival of the French, carried in earth canals and was known as the «Water of the Prophet». It was considered to be especially pure, but it showed an effect of opalescence. Once the canalisation was properly taken care of, there was an apparent decrease of amoebiasis in Morocco.

On the other hand, the importance of the transmission of amoebiasis by the hands, the cysts which are on the hands, which must be an important factor in the transmission of amoebiasis, but there is in drinking water a factor of rapid diffusion which would explain the sudden brutal onset of large numbers of cases of amoebiasis.

SWELLENGREBEL (Amsterdam) — *(translates)* The gentleman who was speaking just now comes from Morocco. That is to say, he doesn't come from Morocco, but from Bordeaux, but he has great experience in Morocco. There he has found that the drinking water supply was very deficient, so deficient that in many instances the water wasn't even clear. It was opaque. Then there was very much amoebiasis among the population. Measures have been taken to improve the water supply, the drinking water supply, and there again this improvement, this hygienic improvement, has greatly reduced, or, to be more correct, has at the same time, or after this improvement has been made, amoebiasis has been greatly reduced.

SCHNEIDER (Paris) — Just a word. One thing strikes me in this academic discussion. We are in a position to count microbes in water in any town and epidemiologists will tell you how many millions or milliards of bacteria he can find in the water. Can anybody say how many amoebas he found somewhere in the water? It would be a very interesting thing to know. We think the water is dirty, it looks like this, and it looks like that, but nobody has been looking under

a microscope to tell us in what town he has been finding amebas in the water. I would like to have an answer to this question.

MORETTI (Bordeaux) — *(translation from tape)* In reply to our compatriot Dr. Schneider, *E. histolytica* has been isolated in Morocco from sewage effluents in Morocco in the classical work of M. Fils St. Marie.

*(Background voices.)*

There was direct communication between this effluent and the drinking water.

SWELLENGREBEL (Amsterdam)—*(translates)* Dr. Moretti answering his predecessor says that in Morocco *E. histolytica* has actually been isolated, by culture of course, from sewage. Now the answer was, «Yes», from Dr. Schneider, «I am not speaking of sewage, I am speaking of drinking water». But answers Dr. Moretti, «Open communication between the sewage and the water supply, so although the amoeba have not been actually found in the drinking water, there have been found in the open, in medium, in a liquid which was in open communication with the drinking water, which is the next best thing that could be done».

BROOKE (Atlanta) — As far as the isolation of *E. histolytica* cysts from water is concerned, a technique has been demonstrated by Chang at the Environmental Health Centre in Cincinnati. Actually whether or not it is practical is another matter, but I don't know whether we are so concerned with trying to count amoebic cysts in water supplies, but what we really need to have is some sort of method for detecting sewage contamination, other than purely a bacterial method which is not effective when you have chlorination of the water supply.

ELSDON-DEW (Durban) — Mr. Chairman, this question of contamination of water supplies by sewage, so well illustrated by the South Bend outbreak, makes you begin to wonder whether it is, in fact, a question of infection with cysts which is spreading the disease or whether perhaps in the faecal contamination we are supplying massive

doses of bacteria of different types and so perhaps changing the intestinal flora.

I think that we are a little too much bound by the old bacteriological idea that if you give a typhoid bacillus, you get typhoid. If you get an amoeba, you do not necessarily get amoebiasis. That I think we have established today. When you are eating sewage, you are probably getting something else, besides the amoeba, which may well be acting as a trigger, either to light up your existing amoeba or to light up the amoebae you happened to have drunk at the same time.

NEGHME (Chile) — I should like to call attention to the isolation of *E. moshkovski* in sewage made by do Amaral in Brazil, and observed also in our country by Weinstein.

HARINASUTA (Bangkok) — *(edited version)* The results of stool examination of the people of Thailand by Sadun, some six years ago, showed an incidence in the general population of 0.3-4.5 % only, but, in our hospital of about 800 beds, we found 11 %, of which 20 % had amoebic liver abscess.

The points are: (1) Why do we have such a low incidence in the general population? Sadun might like to say something. (2) Acute dysentery is common. (3) We see a considerable number of liver abscess.

Is this possibly a different strain of *E. histolytica* which likes to attack the liver? This, I don't know. I would like to know whether conditions in those parts of Africa where liver abscesses are common are the same as in our country, or not.

SADUN (United States)—I would like to point out, on the comment on our findings of only 0.5 % amoebae in Thailand, we had carried out only a single stool examination. The stools were brought to us sometimes several hours after they had been collected. The temperature, the environmental temperature in Thailand is somewhat hot, so I am quite sure that we missed a great deal of otherwise positive stools. Nonetheless, it does appear that the prevalence of amoebiasis is not so very high in the population in the rural areas and, yet, amoebic dysentery seems to be very prevalent. Also, as Dr. Harinasuta

has pointed out, one ought to step into Siderat Hospital at any time and will find several beds occupied by individuals with amoebic liver abscess.

So, whereas I concur with him that there may be some specific factors in the area responsible for this unusual virulence, or apparent virulence, of the amoebae, I would also like to stress again that those figures of 0.5 % are not a true indication of the prevalence of amoebiasis in the population.

## DIAGNOSIS

Brooke (Atlanta) — I didn't realise I was going to be called on to speak and I've already spoken on this subject at this meeting. I think we must all realise that there are values and limitations to every diagnostic procedure. Furthermore, it's probably ill-advised for any group to make any strong recommendations with regard to exactly how the examinations should be done by all laboratories, because not everyone in all of the laboratories would be qualified to perhaps follow the procedures which are recommended. Because, I believe, one of the big problems which we have at the present time is to try to see if there is a serologic test which is capable of, at least, determining invasion. If we could get one, that would show us that the organisms have left the intestinal tract, it would be extremely helpful. I mean, left it, not from the point of going outside, but further on the inside.

Neghme (Chile) — I was very much impressed with the paper by Dr. Stamm. It seems to me he's here in this room. In this he says that the direct examination of a fresh smear was the usual method of diagnosing the amoebiasis in the United Kingdom. In our experience the direct fresh smear examination is a poor method of finding *E. histolytica*. We usually make a stain with a PVA fixative following the procedure introduced at the C.D.C. of Dr. Brooke and Dr. Goldman and also the Telemann modification concentration technique. Presently we have found that if we duplicate an examination of the sediment obtained by this Telemann method (it is very similar to the method of Ritchie). The Telemann is a very old one, and we have used it for over thirty years. We have found that when we duplicate the sediment examinations, that we can raise the figures

of the parasite nearly twice. I shall give only one example. In one sediment preparation, *E. histolytica* was found in 33 %, but in two we have found 66 %.

STAMM (Royal Air Force) — I think the answer to this question as to what method you use, as I said in my paper, depends very much on the person who is using it. I happen to have a good lady, who for the last 20 years has done practically nothing else but look down a microscope at faecal preparations, and if she says there isn't an amoeba there, I defy anybody to find one. She is of a particular psychological disposition, in that she is extremely conscientious and will look down for a very long time, far longer, I am sure, than any of us would, and intelligently. She dislikes concentration methods, it's just her attitude; she has tried concentration methods; she has tried all the concentration methods, and she finds that she doesn't get any better results. She prefers to continue to look down the microscope perhaps rather longer than messing about with faecal alchemy and I think that that is really a matter of taste, and there it is.

BOZICEVICH (National Institutes of Health) — I would like to say something about the serological examination. There is no doubt that almost everyone is doing the stool examination. However, I do feel that the serological examination has a merit, and I think even more so now.

Dr. Brooke mentioned our studies at Wassaic Institute. At this Institute we did some serology, and if we correlate the serological finding with the stool finding, it's really amazing. Now with the direct stool method, that we used at Wassaic, we found an incidence of about 25-26 % positive. But a complement-fixation method we found about 25 % of these so-called carriers gave us positive; however, there was no correlation between the stool findings and the complement-fixation. In other words, some of these gave us negative stool finding, but we found a positive complement-fixation and *vice versa*.

Now when we correlated to the concentration zinc sulphate method, the complement-fixation correlation to it was even worse, because our incidence there was about 64 %. So, actually, we can't go much on that. However, these people were asymptomatic.

Now we have found from our — well, a few — instances that our complement-fixation in the amoebic liver abscess and the so-called liver hepatitis, amoebic liver hepatitis, that, there, we found correlation of about 95 %, in our ulcerative colitis series it was about 40 %. It all depends on what you want to do.

Therefore, from the clinical standpoint, if you have, as again we must emphasise, the clinical symptomatology, if you have a picture simulating amoebiasis and that can be supported by serological tests. I feel it is of more value — well, I'll say it's of value in doing a test.

Now, we have also, as we showed in the meeting the other morning, that we find strain differences, serologically. We usually run two antigens and at times three different antigens, that are obtained from different sources and on some of these we find that with one antigen the serum is positive in other instances, I mean on the same serum, with another antigen it's negative; yet, in a second sample of serum with antigen that was negative by the first, would be positive and the first antigen is negative; well, this confuses us more.

Now, whether it is a strain difference, I do not know. I think it's as bad as small race in *hartmanni*. So we add more confusion to the whole picture. Nevertheless, I feel that once we obtain an antigen that is really a good antigen and one that we can measure — now I don't know, we tried counting amoebae and between three of us we get such variation on the same sample and so forth, that it makes it difficult to even establish what you might say is a good antigen, because you cannot quantitate it.

So, if we obtain a quantitative method in establishing the amount of antigen that we could use to really standardise, then I think that the serology will be improved, and I think that the serological test should be used, because we can follow it.

We've even followed this with cases of amoebic liver abscess where the amoebae were missing from the intestinal tract, but we found them in the liver abscess. Where we followed the treatment, we can show in certain instances that the complement-fixation titre will go down and will become negative. However, in those cases which relapse, the complement-fixation titre never did go down. We have one instance in which we followed that individual for over three years, and he's had three remittances to the hospital.

BLANC (Marseilles) — *(translation from tape)* At this Congress I have heard much more talk of the amoeba, and of amoebiasis, than of the «amibien», but I cannot forget that I am still a doctor. I have always been struck by the difficulty of establishing a diagnosis of amoebiasis, that is to say, to give to each patient who comes a diagnosis satisfying to his conscience as a doctor.

If a patient presents with a leprosy, everybody knows what is meant, but when a patient or a doctor talks of amoebiasis, it is difficult to place this patient in a suitable category. We talk of acute amoebiasis, of chronic amoebiasis. What is chronic amoebiasis? Can we classify together the constipated patient who for the past twenty or thirty years in Europe has had difficulty with his stools without alteration in his general state, together with the repatriate from the war in Viet-Nam who had been passing 40 stools per day for the last year and a half? This latter might, as he has been passing so many stools, be considered as acute, but, as the condition has lasted for years, he might be considered chronic. This is a nosological problem for which no solution has been proposed.

I believe that it is possible to arrive at an adequate precision to put each «amibien» in his place. Amoebiasis may be characterised by its mode of onset whether it is a primary attack or a relapse; it has a particular clinical picture. There is diarrhoea, a dysentery or a dyschezia, or it is characterised by a particular anatomical localisation, it is completely colonic — or confined to the colo-rectum. But in this picture there may also be the hepatic complications.

Thus it is possible on clinical grounds to place each patient in his category. But it is necessary that all medical men talk in the same language. Each patient must be classified in the same way and, more important, in a way understood by all coucerned.

It seems as if we have forgotten the clinical problem of amoebiasis — a serious problem — possibly the most important part of amoebology, because the physio-pathology of amoebiasis is extremely difficult. The role of the parasite, which was formerly a saprophyte depending entirely on the colonic flora in the bowel interior, may suddenly invade the tissue.

There are far more «amibiens» than there are cases of amoebiasis, but until we all talk in clear language we cannot define these.

SWELLENGREBEL (Amsterdam)—*(translates)* I can only summarise the most interesting remarks made by Dr. Blanc. He makes a plea for a clinical approach. All that talk about amoeba, it is all very well, that amoeba, which is after all a saprophyte (I suppose he means a commensal, but that doesn't matter). If a medical man has a patient with leprosy before him, he can classify him according to his clinical symptoms. Well, that is what we have to do also in amoebiasis. We have to ask where are the processes localised, colon, in the sigmoid or in other parts (Oh! in the caecum not to forget). We have to ask his descendants his past history, and in this way patient by patient can be classified in a way so as to arrive at an adequate treatment of that particular patient. If we lose sight of the purely clinical aspects, we are apt to lose ourselves in loose talk to the detriment of the patient himself.

WOODRUFF (London) — I think, Mr. Chairman, that in connection with diagnosis, what we are all really concerned with is the answer to the question — when we are to treat the patient and when we are not.

In that connection, I would suggest that the complement-fixation test is inadequate. If one is to treat patients on the basis of a clinical history and a positive complement-fixation test, one would end up by treating a large number of patients with alimentary neurosis, and the handling of those patients would ultimately become much more difficult, because they have been treated and managed as patients suffering from amoebic infection.

It would be of interest to known, I think, for how long the complement-fixation test remains positive. I wonder whether Dr. Bozicevich would be able to tell us something of that. Is it, for example, something which he finds becomes negative shortly after treatment or does it remain positive for a long period after infection, in which case one might expect the vast majority of people living in endemic regions to have a positive test?

It would again be of limited or no value from the point of view of deciding whether the patient is to be treated. We, in London, have some experience of the complement-fixation test in the alimentary infection. We submitted to the Medical Research Institute a series of patients in whose stools we have found *E. histolytica* or their cysts,

and another series (this is some years ago) of laboratory attendants, and some nurses, and some clerks who had never been out of England, and in whose stools no *E. histolytica* or their cysts could be found and we got about equal results in each series. About 50 % of those who were infected had a positive complement-fixation test and 50 had negative, and in those who were uninfected exactly the same results were obtained.

Rail (Ghana) — Mr. Chairman, the question of diagnosis in these cases of amoebic infestations seems to be intimately bound up, not only in the differentiation of amoeba and their pathogenicity, but also with what the previous speaker but one said a clinical diagnosis. Now, as one of Dr. Elsdon-Dew's diagnostically destitute gentlemen (if I may say so), who can still nevertheless tell an amoeba from a sperm, I feel that I would like to add my weight to the plea for clinical, first clinical, work in the diagnosis of amoebiasis.

We, as physicians, are tending all too much these days to forget that we are physicians, and to hand matters over to the laboratory to make a diagnosis for us. I am afraid that wasn't what God gave us brains for, they were put there to use, and not to look down a microscope. That is the first point that I would like to plead for — the use of your brains in estimating a diagnosis before asking the laboratory to confirm it; not asking the laboratory to make it for you.

Now, the other point I would like to make is one of which is applicable to those of us who work without, and I emphasise this, without the magnificent laboratory facilities which everybody else seems to have here. They only have to take a stool and somebody will sit down and tell you exactly what kind of amoeba it is, and how many it is, and test its pathogenicity for you, and ultimately tell you whether you should treat the patient or not, on the basis of what they find.

Now, we in Ghana run on, I would say, a much more simplified laboratory system than this. We have a pathologist, and under him we have trained and semitrained technicians who, once they are able to differentiate *E. histolytica* from *E. coli,* are then regarded as sufficiently capable of going out to the smaller hospitals where they will have to aid the medical officer in doing his work.

I feel that if this meeting could lay down something quite simple, whereby the lesser pathologists and bacteriologists could work and

gain information to help their doctors, and yet preserve themselves the right to go on developing their species, breeding their cultures, and developing their serum reactions. We, in the days when I learned to differentiate amoebae, had one basic governing rule. If the amoebae had red cells in it, it was pathogenic; if it hadn't, it wasn't.

CHAIRMAN — Would you please make your communications as short as possible.

MORETTI (Bordeaux) — *(translation from tape)* I am entirely in agreement with Dr. Blanc in stressing the importance of the clinical approach, and I hope that everyone at this Congress will go away with this in mind.

I want to talk particularly of the difficulty confronting the discussion in the differential diagnosis of the «amibien». During this Congress we have not heard much of the most frequent presentations of amoebiasis and I must say that the most frequent presentation is a false amoebiasis.

Perhaps the patient may have an ulcerative colitis or possibly a cancer of the rectum which present us an emetineresistant amoebiasis. I would have liked to hear more of the differential diagnosis of these at this Congress.

Then we come to the question of eosinophilia in amoebiasis: for all French colonial physicians, when there's an eosinophilia, whether in the blood or in the stool, this eosinophilia is associated with helminths, with worms. Even if we find the amoebae, we do not associate the eosinophilia with the amoebiasis, but to a verminous parasitosis which we may or may not be able to discover. I feel that the eosinophilia in the stool is due to the blood which is passing into the stool during the dysentery process.

SWELLENGREBEL (Amsterdam) — *(translates)* Dr. Moretti insistis on what we might call the differential clinical diagnosis. In Bordeaux there are numbers of patients diagnosed, or at least in the beginning diagnosed, as suffering from amoebic dysentery, and, then, it turns out that they are either suffering from ulcerative recto-colitis or cancer of the rectum. These are two of great importance, which very much leads one astray.

Furthermore, he also insists on the eosinophilia, not only on the eosinophilia of the blood, but also on the eosinophilia of the blood which is mixed with stools. It is taken for granted, and as a rule is correct, that eosinophilia is due to some helminthiasis, but he wants to know whether that is really true and whether perhaps eosinophilia is not also, could not also be associated with amoebiasis.

SWELLENGREBEL (Amsterdam) — I do not want to speak of the diagnosis of amoebiasis, but simply I want to speak about how to find amoebae in any form in the stools, as it is done in my laboratory. I have not a magnificent laboratory, but the only thing I have is a magnificent technician. That's true. Now we have to take apart two different things, finding amoebae in dysenteric stools; in the mucus. When we fish a little bit of mucus out of the stool and there we shall find the *histolytica* stage of the amoeba. That is easy to recognise, it is the large size of the amoeba, the way they emit their pseudopodia and the presence of erythrocytes in the amoebae. In the living, in the moving amoeba, it produces wonderfully big pseudopodia, but if there are no erythrocytes, at least not one erythrocyte in one of the amoeba we find, then we will not conclude that it is the *histolytica* stage of the amoeba. But if, at least, in some of them erythrocytes are present, then our diagnosis, our aetiological diagnosis, mind you, not our clinical diagnosis, is certain.

But that's not the whole story. We may also have to find amoebae in perfectly normal stools, or at least apparently normal stools. Even in these stools, we may even find actual *histolytica* amoebae. A method which for the last 40 years in my laboratory has always proved very useful, and which you will not find in Dr. Brooke's book, is what we call the «eosin» method. It is extremely simple, it is simply based on the fact that eosin is a dye which does not stain living tissue or a living cell. So, if we mix a small amount of the stool to be examined with a 2 % eosin solution, then everything which is living will show as white discs on a rosy background.

You can look for them at low magnification, but, of course, if you find something, you will have to apply a higher magnification — not oil immersion, but simply a dry higher magnification. It may be anything; it may even be a blastocyst, but in this way it is much easier to find cysts than in any other way.

Amoeba at that stage, unless it is a real *histolytica* stage, are of no use, because you can never be quite sure that the amoebae you find, whether it is a *minuta* or whether it is a *coli,* whether it is even a *nana,* so leave the amoeba alone completely and concentrate on the cysts.

In such a preparation, such a living preparation, it is easy to see the chromidia, but, then, it is not easy to see the nuclei, then you have to make a second preparation, and that preparation will be a preparation in lugol's solution, a solution of iodine, a weak solution of iodine; there you will see the vacuoles, the glycogen vacuoles. You will see the nuclei, and so in the case of *E. histolytica* you will see a uni-nucleate cysts with a big glycogen vacuole. If it is *coli,* you will see that the glycogen vacuole is present in the bi-nucleate cysts. These are all very simple methods which can be carried out in any laboratory of course; you can also apply both Telemann concentration.

*(Time expired.)*

There was no time for discussion of the NEED FOR THERAPY.

CHAIRMAN — Now, as Chairman, I have to ask for specific recommendations.

It seems to me, listening to this discussion, that we've all agreed that we don't really know what we are talking about. No one has been able really to define what he means by *histolytica,* no one has defined what virulence is, or what pathogenicity is.

I would finally like to stick my neck out, and suggest the definition of virulence and pathogenicity. If we accept Dr. Swellengrebel's idea that the invasiveness is ultimately inherent in the organism, in one organism and not in another, then could we use virulence as a term meaning the possibility of invasiveness which is hereditary in the organism of which we are dealing. Then could we define our use of the word *pathogenicity* to describe an invasion, possibility of invasion which takes place when a certain particular amoeba meets a certain particular host under certain particular conditions.

Now, the recommendations will be started off by Dr. Elsdon-Dew.

## GENERAL DISCUSSION AND RECOMMENDATIONS

ELSDON-DEW (Durban) — I think the position is quite obvious. For a start, I don't think at any time we would ever get together as many people who are interested in amoebiasis, and any recommendations or resolutions passed by a collection such as this is bound to have some impact on the disease all over the world.

The first thing that strikes me is that different parts of the world do not appreciate the amoeba properly. What I'm getting at is in some parts of the world the amoeba is considered always to be pathogenic, in other parts of the world — the more enlightened ones if I may say so — the amoeba is regarded sometimes as a commensal.

But there is no simple guiding theme to this and what I would like to suggest to this meeting is that we ask some central organisation to set up an assessment of the incidence of the disease in various parts of the world, the parasite coming second, so that the single organisation could act as a guide to the relative importance of amoebiasis in any one area. I think we need to do a lot of education. I have the motion written here; I would like to have your comments on it.

CHAIRMAN — Would you like to put the motion as such?

ELSDON-DEW (Durban) — I'll put the motion: «An assessment of the incidence of the disease and of the parasite should be carried out in various parts of the world by some central organisation.»

ELSDON-DEW (Durban) — I will modify it according to Dr. Neghme's suggestion if that meets with your approval: «An assessment of the incidence of the disease and of the parasite should be carried out in various parts of the world under the auspices of some central organisation.»

CHAIRMAN — You have heard the motion; those in favour? Against? Carried! Any other recommendations?

In the general discussion which followed, DR. BEAVER suggested that the related and confusing conditions, such as *Trichuris* dysentery and ulcerative colitis, should be incorporated.

DR. BROOKE suggested that evaluating services be set up so that results would be comparable. DR. JACOBS suggested that serology should form part of the scheme.

CHAIRMAN — What bothers me about these recommendations is what are we going to do with them. If we suggest that some central authority should do these things, which are highly desirable. I think we ought to try to decide what central authority we are talking about, because, if we don't, this will get no further than it has got at present. I think the suggestion was made that we should consult the World Health Organisation. Would that be agreeable to the meeting as a starting point that the Secretariat of this *ad hoc* meeting should pproach the World Health Organisation just to ask them whether they would regard this as a possible procedure?

UNIDENTIFIED VOICE — I second the motion.

CHAIRMAN — Carried.

ELSDON-DEW (Durban) — Mr. Chairman, may I finally say a word? I think that one thing that we have to do is to thank our Chairman for his very able conduct of what, I feel, has been a very sucessful meeting.

## ATTENDANCE REGISTER

*Alimurung, Mariano M.*, Manila, Philippines.
*Armengaud, M.*, Dakar, A.O. F.
*Azar, G. E.*, Beirut, Lebanon.
*Beaver, Paul C.*, New Orleans, U.S.A.
*Berberian, D. A.*, Rensselaer, U.S.A.
*Blanc, F.*, Marseille, France.
*Bozicevich, John*, Bethesda, U.S.A.
*Brooke, M. M.*, Atlanta, U.S.A.
*Brossi, Arnold*, Basel, Switzerland.
*Burrows, Robert B.*, Tuckahoe, U.S.A.
*Carneri, Ivo de*, Pavia, Italia.
*Chaudhuri, R. N.*, Calcutta, India.

*Devakul, K.*, Bangkok, Thailand.
*Elsdon-Dew, R.*, Durban, South Africa.
*Franco, L. T. de Almeida*, Lisbon, Portugal.
*Frye, William W.*, New Orleans, U.S.A.
*Giudocelli, P.*, Marseille, France.
*Gonnert, R.*, Elberfeld, Germany.
*Gunders, A. E.*, Israel.
*Hill, J.*, London, England.
*Harinasuta, T.*, Bangkok, Thailand.
*Hirsch, K.*, Haifa, Israel.
*Hirsch, W.*, Haifa, Israel.
*Jacobs, Leon*, Bethesda, U.S.A.
*Joe, Lie Kian*, Djakarta, Indonesia.
*Jung, R. C.*, New Orleans, U.S.A.
*Kessel, John F.*, Los Angeles, U.S.A.
*Kirsche, D.*, Dakar, A.O.F.
*Koenig, Karl*, Leverkusen, Germany.
*Laarman, J. J.*, Leiden, Holland.
*Lamy, Louis*, Paris, France.
*Maegraith, Brian*, Liverpool, England.
*Meerovitch, E.*, Montreal, Canada.
*Moretti, G.*, Bordeaux, France.
*Mousa, A. H.*, Cairo, Egypt.
*Neal, R. A.*, London, England.
*Neghme, Amador*, Santiago, Chile.
*Nelson, E. Clifford*, Vancouver, Canada.
*Nauenberg, Erna*, New York, U.S.A.
*Otto, Gilbert F.*, Chicago, U.S.A.
*Piganiol, G.*, Dijon, France.
*Rail, G. A.*, Accra, Ghana.
*Roach, G. G.*, Durban, South Africa.
*Sadun, H. Elvio*, Tokyo, Japan.
*Schneider, J.*, Paris, France.
*Stamm, W. P.*, England.
*Swellengrebel, N. H.*, Amsterdam, Holland.
*Thompson, Paul E.*, Detroit, U.S.A.
*Waks, José*, Buenos Aires, Argentine.

*Walters, J. H.*, London, England.
*Wilmot, A. J.*, Durban, South Africa.
*Wolthuis, F. U.*, Amsterdam, Holland.
*Woodruff, A. W.*, London, England.
*Yexikomshian, H. A.*, Beirut, Lebanon.
*Zuidema, P. J.*, Amsterdam, Holland.

SUBSECTION 3

## LEISHMANIASIS

1ST SESSION — **Kala-azar and cutaneous leishmaniasis**

*Monday, September 8th — 3:00 to 4.30 p.m. Room C*

The meeting opened under the chairmanship of Dr. Saul Adler and the Rapporteur was Dr. Samuel Pessoa. The Secretary was Dr. Maria do Pilar F. Gomes.

The following papers were presented and discussed.

## KALA-AZAR IN THE SUDAN AND TROPICAL AFRICA

M. H. SATI

Kala-azar is endemic in East, West, North and Central Africa, in Tunisia, Morocco, Algeiers, Libya, Egypt, Sudan, Abyssinia, Eritrea, Kenya, French Equatorial and West Africa (Niger Territory, Cameroons, Dakar and Lake Tchad Territory). Nigeria and Portuguese East Africa (Mozambique and the Somali land).

The endemicity varies from one country to another and from place to place in the same country. In the Sudan and Kenya, in recent years, this disease has attained epidemic proportions and consequently assumed paramount public health importance. In some of these countries, the disease is of such rarity that only a few cases are encountered every year. In the countries of West Africa, on the whole, the cutaneous leishmaniasis seems to be more prevalent than the visceral type. From Egypt, Phillips (1904) reported two cases of kala-azar after which no reports of cases were forthcoming from that country, but it is apparent that the cutaneous type exists in it in several foci, Hallawani (1940) and Sallam (1940).

From Nigeria, Jellife (1955) reports the scarcity of leishmaniasis and reviews its annual reports for several years, elucidating the paucity of cases of both the cutaneous and visceral types.

Before the Second World War, however, there was little information about the existence of kala-azar in Kenya and the unanimity of opinion is in favour of its absence, except perhaps a few cases in its northeastern corner. Since the war, several workers reported its existence in that country (Tobias, 1941 and 1941*a*; Cole, 1942; Cole *et al.*, 1942; Erasmus, 1942; Clinton Manson-Bahr, 1942; Anderson, 1941 and 1943).

Fendall (1952), in a comprehensive review of the whole situation of kala-azar in East Africa, sums up the position in Uganda, Tanganyika and Zanzibar; as no information is available concerning Portuguese East Africa, he quotes Prates (1928) as describing what appears to be the first case of kala-azar south of the Sudan.

Heisch (1954), describing an epidemic of 3,000 cases in Kenya, states that since 1941 cases have been reported from three main localities: Saricho, on the Uaso Nyiro river, in the Northern Province (Heisch, 1947); Ngomeni, between Kitui and the Tana river (Fendall, 1950, 1951); and Machakos district. He comments that the disease is probably more widespread in its distribution and other places suspected in the Northern Province comprise Archer's post (also on the Uaso river), Garba Tulla, Titu and Graissa. He rightly regarded this epidemic in Kenya as the first of its kind in Africa.

From Eritrea, Ferro-Luzzi (1943) reported 9 cases seen over a number of years and stresses the scarcity of cases. Although reports from Ethiopia are scanty, Anderson (1943) incriminates the Omo river, Nairobi-Addis Abbaba road and Southern Abyssinia. The Western Abyssinian frontier is infected (Kirk, 1939).

Since Neave (1904) reported the first case of kala-azar from the Sudan, several workers studied the disease, among whom there were two Government Commissions, one under Bousfield (1908) and the other headed by Thomson (1911). All workers in the Sudan were in agreement about the paucity of cases and their erratic distribution but for a few small outbreaks, that cropped up from time to time.

These outbreaks were uniformly consequent upon patrolling in uninhabited country or in isolated military posts: at Jongols post and Kurmuk (Archibald, 1923) and at Wad Arud (Kirk, 1939). Stephenson (1940) described the first outbreak recorded amongst the civil population (the Dinkas of Melut). It is note-worthy that the recent epidemic (1956) included these areas, that remained silent for

a considerable number of years, almost a quarter of a century in the case of Kurmuk and Jongols post area.

## AREAS OF THE EPIDEMIC

In the Fung, the disease used to be mainly along the Blue Nile and its tributaries, more round Singa, Sennar and north of Roseiries. There were practically no cases in the Southern Fung and specially areas away from the river. The disease used to be prevalent along the river Dinder. In Upper Nile the disease lately became endemic in Melut and its neighbourhood on the White Nile and its branches. This focus was very much localised. The area between this and the Abyssinian frontier is a waterless plain in the dry season, while it is a water-logged, swampy region in the wet season.

In this epidemic, however, the area between the Blue and White Niles right from the Abyssinian frontier to the railway line joining Sennar (on the Blue Nile) and Kosti (on the White Nile) and beyond it to varying depths and past the White Nile in the Southern reaches has been affected. Here it crosses the White Nile and sporadic cases disseminating from the area of the epidemic had been discovered in the Nuba Mountains and other places in Kordofan. The Sennar-Kosti railway line has now been broken through at various places; besides the break through at Sennar, another one at Kosti itself and, going north of it, at Gezira Abba is reported. The White Nile was, in the recent past, free from the disease, except the focus at Melut described by Stephenson. This focus has now joined with that on the Ethiopian border as all the area intervening had been now infected. Areas on the west bank of the White Nile as far north as Kaka and going right west into the Abu Gebeiba area is now part of the endemic area.

It has to be stressed that this epidemic hit hard primitive tribes in the Southern Fung and Upper Nile areas, at or near the military posts, that produced small outbreaks in the past and referred to above. Tribes affected are Jum Jum, Surkum, Maban, Uduk and Dinkas.

## AETIOLOGICAL FACTORS

Several factors are involved in the causation of this epidemic, some of which are known while others are unknown. The following observations are considered relevant because they may have a direct or indirect bearing on the problem under discussion.

1. *Economic development.* — Economic development in the Northern Fung and Renk district has brought many non-immunes to the endemic area either as cultivators or cotton pickers. Some of these contracted the disease and went back home, importing infection to new areas. In both areas on the White and Blue Niles pumping schemes with big cotton cultivations had been established, with thousands of newcomers to the kala-azar endemic area. This state of affairs bears resemblance to the Tea Estates of Assam in the 10th century.

2. *Improvement of communications.* — Since 1953 the Southern Fung became connected to the remainder of the country by a railway line. Pumping schemes in the Renk district improved and increased motor transport.

3. *The mass movement.* — During the time of the Condominium Government these areas were closed, but since Independence free ingress and egress is the rule as all such barriers were lifted.

4. *Nomads.* — The seasonal wander of the nomadic Arabs, Fellatas and Dinkas, no doubt, contributes to the spread of diseases. In this particular epidemic it is evident that the infection has been brought from the Northern Fung to the Jum Jum, through the agency of the Arabs of the Rufaa El Hoi tribe, who come to the Southern Fung for grazing their cattle during the dry season, as well as by the Jum Jum shepherds who were employed by the Arabs for the first time and taken to the Northern Fung, where the disease lately has epidemic spread.

5. *Primitive tribes.* — The disease having affected such backward tribes has not been notified in time, not until it spread and became a real epidemic.

6. *Loss of cattle before the epidemic.* — In both areas there was a heavy loss of cattle through pleuro-pneumonia and foot and mouth disease. Unconfirmed reports say that there was also death amongst

wild game before the epidemic. Insects and sandflies switched on to humans on finding no cattle to feed upon.

7. *Rainfall.* — Two years before the epidemic the rainfall was moderate, but the third year before the rains were bad. The year of the epidemic the rainfall had been exceptionally heavy and so was malarial incidence.

8. *Gum tappers.* — These people come from the non-endemic area for tapping and collection of gum. These non-immunes contract the disease and carry the infection to their homes, and so they help the incidence of the disease if other conditions are favourable for its spread-vectors, etc.

9. *Mechanisation in crop production in Gedaref area (Kassala province).* — This has resulted in the cultivation of over 250,000 acres in the rain-cultivation and is attracting thousands of labourers and cultivators, the majority of whom are non-immune. Therefore, an epidemic is imminent (Kassala).

## AGE AND SEX DISTRIBUTION

Please, see Table 1.

## IMMUNITY IN KALA-AZAR

Immunity in protozoal infections is still imperfectly understood, although the general principles of immuniology do apply. There are, however, certain interesting features of kala-azar in the Sudan, which invite speculation and elucidation. This certainly applies in the case of the comprehension of the mechanism of immunity.

One of these interesting aspects is the classification of kala-azar into a primary, secondary and tertiary manifestations (Kirk, 1944), which are, in fact, milestones in the evolution of immunity of this disease, which depends on several factors, the most determinating of which are the virulence of the organism and the individual resistance as well as the presence of other infections and complications.

There is also this question of latency of the disease (Wenyon, 1926) and its activation by battle stress, malaria, relapsing fever

(Corkill, 1948 and 1949). There is the inherent assumption that the infection must be hiding somewhere in the body and therefore capable of being activated. Another interesting feature presented itself in the recent epidemic of the presence of leishmania in the lymph glands without the manifestations of the disease in several school children in the epidemic area.

TABLE 1

| Sex | Number examined | Gland puncture L.D.B. positive | Enlarged spleen | Enlarged glands Lymph glands |
|---|---|---|---|---|
| Boys . . . . . | 221 | 18 | 4 | 18 |
| Girls . . . . . | 175 | 2 | — | 2 |

In another school the result is as follows:

TABLE 2

| | Number examined | Gland puncture L.D.B. positive | Enlarged spleen | Enlarged glands Lymph glands |
|---|---|---|---|---|
| Boys . . . . . | 146 | 23 | 8 | 20 |

The presence of parasites in the lymph glands is significant of a post-primary, aberrant, abortive or latent stage in the evolution of the disease. It seems reasonable to suppose that this can be activated by stress, malaria, etc., at least in some cases. Before the war the writer had been under the impression that certain cases with splenomegalies but no symptoms had been suffering from kala-azar, but they were persistently negative spleen and sternal puncture; at that time the gland puncture had not been evolved as a method of diagnosis. A few years later some of these came with kala-azar. Whatever the explanation of this manifestation of the disease is, it seems that it is a state of equilibrium in the host-parasite relationship analogous to premunition in malaria. It can be called therefore «premunition in kala-azar».

In the literature, 2 cases of lymphatic enlargement due to infection with leishmania in two young otherwise healthy American servicemen have been reported by Angevine *et al.* (1945). While writing, Bell *et all.* (1958) described a similar thing from Cyprus and Malta, also among young servicemen, under the title «localised leishmaniasis of lymph nodes». They reported 4 cases and the lymphatic enlargement was in the cervical region in 3 cases and in the axillae and groin in 2 and it was femoral in 1 only. All were otherwise healthy. This means that this syndrome is commoner than we think and merits attention. With the gland puncture technique many more cases will be discovered.

## TRANSMISSION AND SEARCH FOR ANIMAL RESERVOIR HOST

In the Sudan, Kirk and Lewis carried out an intensive study of the problem of sandflies and kala-azar. They studied sandflies and published a series of very interesting papers. In a review of this question Kirk and Lewis (1955) suggested that *P. orientalis* may fill the criteria in some parts of the Sudan. They maintain that other sandflies related to those that transmit kala-azar in other parts of the Old World and occuring in the Sudan are *P. leslyae* and *P. martini,* but they had not carried any feeding experiments with any of these. They found *P. clydei,* although not belonging to the group of phlebotomus by which kala-azar is transmitted in the Old World, agrees fairly well in its distribution with kala-azar and has been found to bite man.

In the recent epidemic of kala-azar in the Sudan, Qutub'Din (1957 and 1958) encountered none of these sandflies in the area of the epidemic, except a few *P. orientalis,* collected from Melut, as well as *P. clydei,* from the same place

Recently Heisch (1954), in the last epidemic in Kenya, found a few *P. orientalis* in the northeastern part of the country, but none of others described by Kirk and Lewis in the area of the epidemic. Heisch suspects new species as vectors in the last epidemic in Kitui. In the Sudan it is realised that more collections are to be carried out in the area of the epidemic. Heisch considers *P. clydei* as potential vector in spite of the fact that he failed to find it in the new epidemic area.

Kirk and Lewis (1955) remark: «although we had not been able to unravel the whole story, a considerable amount of information has been obtained which may be of interest to other workers, especially in view of the outbreak of kala-azar in East Africa during and since the war.»

Malone and Brooks (1944) went as far as to bring a case against the sandfly as vector or kala-azar. The present writer does not by any means question the veracity of the sandfly theory, as the evidence is overwhelming in favour of the sandfly, but feels that there are more factors involved which merit attention and further study and in this connection he is in agreement with de Azevedo (1948) when he says: «in spite of the general acceptance of the *Phlebotomus* as vector based on experimental work, nearly all those who have made epidemiological studies have come across factors difficult to explain on the assumption that transmission by the phlebotomae is the only means of disseminating the disease.»

In the Sudan it has been observed that the nomadic Arabs leave the kala-azar endemic areas during the wet season, from June to December, to avoid the attacks of tabanidae on their cattle. This is the case with several tribes, 4 or 5 big tribes in the Fung, where the epidemic occured. In the endemic area, during the wet season, the stable-fly as well as other tabanidae do attack man. These in this area transmit animal trypanosomiasis and hence the wander of the nomads to desert areas. Their distribution coincides with that of kala-azar. In view of this and the fact that Berberian (1938) could effect mechanical transmission of oriental sore through the agency of stomoxys calcitrans, it is suggested that this fly may be of importance in mechanical transmission of kala-azar at least in times of epidemics. In the absence of animals these flies attack man for their feeding. In summer these insects abound near water courses and rivers, where usually kala-azar flourishes.

## ANIMAL RESERVOIR HOST

The search is continuing on the same lines as described by Kirk (1956), but no positive findings were obtained in this epidemic.

## DIAGNOSIS

In this epidemic the gland puncture (Kirk and Sati, 1940) has established itself as a reliable method of diagnosis which is safe and simple and does not need any preliminary preparation and carries no risks to life. It has advantage over other methods in disclosing such cases that are positive from the lymph glands without symptoms, as described above. The following shows the percentage of positive by the spleen and/or gland puncture in the different centres in this epidemic.

TABLE 3

| Place | Number examined | Positive gland | Percentage | Positive spleen | Percentage |
|---|---|---|---|---|---|
| Wadeka . . . . . | 58 | Not done | — | 32 | 55 |
| Gelhak . . . . . | 109 | 46 | 42.2 | 39 | 35.7 |
| Ingassana Hills . . . | 62 | 37 | 59.6 | Not done | — |

From the above you will see that the gland puncture is as effective as the spleen puncture and may be superior to it. Its superiority lies mainly in the fact that it can be done in the field in mass surveys and any body can do it.

## TREATMENT

The drug exlusively used in the treatment of kala-azar in this epidemic is pentostam. The majority of the cases were treated in their own villages, under very adverse conditions. The total number treated was 5,577 cases; of these, 718 cases only were treated in hospital with 40 deaths and a percentage of 5.6. This excells any previous result from the Sudan for hospital cases. Kirk and Sati (1940) had an immediate curerate of about 82 % with the daminides, while Sati (1942) recorded the best results in the Sudan with antimony with an immediate cure-rate of 77 %.

When Kirk and Sati (1947) remarked about pentostam «it may be possible in the future to treat selected cases in the out-patient

department», it was considered by some of our colleagues too good to be true in view of the resistance to treatment of Sudan kala-azar and the high death rate in spite of the meticulous nursing care.

Clinton Manson-Bahr and Heisch (1956) comment that the disease round Kitui, in Kenya, seems to be more resistant than the Sudan type, which is easily cured by pentostam. It seems that it is more a question of difference in dosage, as they gave their cases a total of 2 g to children and 4 g to adults; in this last epidemic a minimum of 6-8.4 g was given regardless of age. No toxicity whatsoever has been encountered. Even with this dosage, a relapse rate of 1-2 % has been recorded.

Several cases relapsed after a second course of pentostam; a few others could not be cured after the third course. Frank resistance to antimony had been encountered and lomidine had been resorted to with satisfactory results. The dosage of lomidine is given in 2-3 mg/kg body weight. The resistance of the strain in this epidemic is obvious as in the past 3.6 g. Pentostam used to cure a case of kala-azar in the Sudan and very rarely in a limited number of cases 4.8-6 g were needed.

TABLE 4 — *Sample of 1280 cases showing the distribution of the disease amongst men, women and children*

| Province | Place | Men | Women | Children (both sexes) |
|---|---|---|---|---|
| Blue Nile. . . . . | Wadeka | 74 | 82 | 153 = 309 |
| Blue Nile. . . . . | Regreig | 5 | 1 | 11 = 17 |
| Blue Nile. . . . . | Ulu | 4 | 14 | 11 = 29 |
| Upper Nile . . . . . | — | 294 | 206 | 425 = 925 |
| *Total* . . . . | — | *377* | *303* | *600 = 1,280* |

## SUMMARY

Kala-azar is endemic in North, West and East Africa. The degree of endemicity varies from one country to another and in different localities in the same country. The disease is becoming of public health importance in both the Sudan and Kenya and, in recent years, attained in them epidemic proportions. In the past half century after its discovery by Neave (1904) in the Sudan, the disease had

been characterised by its paucity and erratic distribution, but for a few small outbreaks in military posts. In 1956 a violent epidemic of an unprecedented scale blew up in areas that used to be loosely endemic. This hard hit primitive tribes, in whom the infection had not been known to occur in the recent past. Short description and analysis of this epidemic had been attempted and suggestion is adduced that mechanical transmission may play a role in epidemics, with special consideration to tabanidae in general and stomoxys calcitrans in particular. *Phlebotomi, orientalis, leslyae* and *clyedi,* suggested by Kirk and Lewis as probable vectors in the Sudan, had not been encountered in the areas of the epidemic but for a few *P. orientalis* collected from Melut in April, 1957. The search for animal reservoir host continues. The usefulness of the gland puncture in this outbreak is stressed, especially in mass surveys. During the survey, a state of equilibrium in the host-parasite relationship, which was compatible with normal health, had been encountered. This is analogous to premunition in malaria. With regard to treatment pentostam had been an effective drug, although it seems that the strain in this epidemic is more resistant than previously. A relapse rate of approximately 1-2 % had occured. These reacted to limidine (pentamidine).

## BIBLIOGRAPHY


1. Anderson, T. F. — *East African, Medic. J.,* 1941.
2. Anderson, T. F. — *East African Medic. J.,* xx, 172, 1943.
3. Archibald, R. G. — *Amer. J. Trop. Med.,* iii, 307, 1923.
4. Angevine, D. M., Hamilton, T. R., Wallace, F. G., and Hazard, J. B. — *American Journal Medical Society,* **210** (1): 33-38, 1945.
5. Bell, D. W., Carmichael, J. A. G., Williams, R. S., Holamn, R. L., and Stewart, P. D. — *B. M. J.,* March 29th, p. 740, 1958.
6. Berberian, D. A. — *Proc. Soc. Experimental Biol. and Med.,* **38** (2): 254-256, 1938.
7. Bousfield, L. — Third Wellcome Report, Wellcome Research Laboratories, Baillaire, Tindall & Cox, 8 Henrietta Street, Covent Garden, London, 1908.
8. Cole, A. C. E. — *E. A. M. J.,* xix, 199, 1942.
9. Cole, A. C. E., Cosgrove, P. C., and Robinson, G. — *Trans. R. Soc. Trop. Med. & Hyg.,* xxxvi, 25, 1942.
10. de Azevedo, J. F. — *An. Inst. Med. Trop.,* **5**: 269-287, 1948.
11. Corkill, N. L. — *Ann. Trop. Med. & Paras.,* **42** (2): 224-229, 1948.
12. Corkill, N. L. — *Ibid.,* **42** (2): 230-235, 1948a.
13. Corkill, N. L. — *Ibid.,* **43**: 261, 1949.
14. Erasmus, J. F. P. — *South Africa Med. J.,* xvi, 379, 1942.
15. Fendall, N. R. E. — *E. A. M. J.,* **27**: 291, 1950.
16. Fendall, N. R. E. — *E. A. M. J.,* **28** (2): 62-65, 1951.
17. Fendall, N. R. E. — *J. Trop. Med. and Hyg.,* **55**: 193, 220, 245, 1952.
18. Ferro-Luzzi, G. — *Bol. Soc. Ital. Med. Ig. Trop.,* 1135, 1943.
19. Hallawani, A. — *J. Egyptian Med. Assoc.,* **23** (4): 192-198, 1940.
20. Heisch, R. B. — *East. Afr. Med. J.,* xxiv, 3, 1947.

21. HEISCH, R. B. — *Tran. R. Soc. Trop. Med. & Hyg.*, XXXI, 179, 1954.
22. JELLIFFE, R. S. — *West African Med. J.*, **4** (2): 92, 1955.
23. KIRK, R. — *J. R. Soc. Trop. & Hyg.*, **32**: 271, 1939.
24. KIRK, R. — *Ibid.*, **38**: 61, 1944.
25. KIRK, R. — *Ibid.*, **50** (2): 169, 1956.
26. KIRK, R., and LEWIS, D. J. — *Ibid.*, **49** (3): 329, 1955.
27. KIRK, R., and SATI, M. H. — *Ibid.*, **33**: 501-506, 1940.
28. KIRK, R., and SATI, M. H. — *Annals Trop. Med. & Paras.*, **35** (1): 34-37, 1943.
29. KIRK, R., and SATI, M. H. — *Ibid.*, **41** (1), 1947.
30. MALONE, R. H., and BROOKE, A. G. — *Indian Med. Gaz.*, **79** (10): 484-493, 1944.
31. MANSON-BAHR, C. — *Lancet*, **1**: 217, 1942.
32. MANSON-BAHR, C., and HEISCH, R. B. — **50** (465), 1956.
33. NEAVE, S. — *B. M. J.*, **1** (1252), 1904.
34. PHILLIPS, L. — *J. Trop. Med.*, August 1st, 1904.
35. PRATES, M. — Comunicado ao Congresso de Medicina Tropical do Cairo, December, 1928.
36. QUTUB'DIN, M. — Report to the Director, Ministry of Health, 1957.
37. QUTUB'DIN. M. — Report to the Director, Ministry of Health, 1958.
38. SATI, M. H. — *Am. Trop. Med. & Paras.*, **36**: 1, 1942.
39. STEPHENSON, R. W. — *Annals Trop. Med. Paras.*, **34**: 176, 1940.
40. SALLAM, HAMED — *Laboratory & Med. Progress Cairo*, **1** (2): 164-165, 1940.
41. THOMSON, D. S. B., and MARSHALL, W. E. — Kala-azar Commission. Forth Wellcome Report, Baillaire, Tindall & Cox, 8 Henrietta Street, Covent Garden, London, 1911.
42. TOBIAS, R. I. — *E. A. M. J.*, XVIII, 336, 1941.
43. WENYON, C. M. — Protozoology, Baillaire, Tindall & Cox, 8 Henrietta Street, Covent Garden, London, 1926.

# ÉPIDÉMIOLOGIE ET PROPHYLAXIE DE LA LEISHMANIOSE VISCÉRALE HUMAINE (KALA-AZAR) MÉDITERRANÉENNE

P. GIRAUD

## ÉPIDÉMIOLOGIE

Si la thérapeutique de la leishmaniose viscérale humaine est entrée dans la voie des réalisations presque parfaites avec l'avènement des nouveaux produits stibiés et des diamidines, par contre les modalités de transmission de la maladie sont loin d'être encore parfaitement connues. Et cependant, nous ne pourrons instituer une prophylaxie efficace que le jour où nous connaitrons de façon certaine les donnés étiologiques.

Nous avons personnellement étudié de très près les conditions de l'épidémiologie en France depuis 1922 et nous pouvons dire que nos constatations concordent à très peu de choses près avec celles des auteurs qui ont étudié la maladie de ce point de vue dans toute la région méditerranéenne.

Il semble bien que les conditions d'éclosion du kala-azar et sa propagation soient les mêmes en étendant la zone qui nous occupe non seulement à tous les pays riverains de la Méditerranée, mais encore aux bords de la Mer Noire et aussi à toute la Péninsule Ibérique, Portugal compris.

Par contre, il semble bien que des différences notables soient observées en ce qui concerne le kala-azar Indou et des conditions de race, de climat et aussi les conditions locales d'hygiène et les variations de la forme des insectes vecteurs interviennent sans doute pour motiver ces différences.

Nous développerons nos idées à ce point de vue au cours de cet exposé.

Par contre, nous n'avons pas encore une connaissance suffisante de l'épidémiologie de la leishmaniose viscérale en Amérique pour avoir une opinion précise à son sujet et nous laissons à nos collègues Brésiliens, dont les travaux sont importants à ce point de vue, le soin de nous éclairer sur les analogies et les discordances que l'on peut observer par rapport au kala-azar méditerranéen dans leur continent.

## ÉVOLUTION DE L'ENDÉMIE

*L'endémie humaine de la région marseillaise est à peu près stable depuis 1922,* époque à laquelle nous avons diagnostiqué le premier cas, dans le service de notre Maître, le Prof. d'Astros.

Peut-être la maladie existait-elle auparavant, mais il semble bien qu'elle ait, en tous cas, subi une recrudescence manifeste aux environs de cette date.

Quoiqu'il en soit, on peut fixer à 10 cas par an environ le nombre des malades que nous observons à la Clinique Médicale Infantile. Des différences assez importantes peuvent être observées suivant les années, en particulier une chute spectaculaire pendant les années de guerre (1942-43) sans aucune prophylaxie spéciale, bien au contraire, puisque ce furent pour nous des années de misère et de forte mortalité infantile. Mais dans l'ensemble la courbe est tout à fait stationnaire et nous en sommes à 346 cas à l'heure actuelle après 35 ans d'observation.

La fig. 1 objective la stabilité de cette endémie.

## AGE DES SUJETS ATTEINTS

Comme lors de nos statistiques antérieures, nous trouvons une très forte prédominance de cas dans la petite enfance et tout particulièrement de 1 à 3 ans.

Dans la première année les cas sont rares: 13 cas seulement. Le plus jeune avait 6 mois, un autre 7 mois au moment du diagnostic. Cette immunité relative des nourrissons contraste avec l'atteinte massive des enfants de 1 à 2 ans, 90 cas, et de 2 à 3 ans, 88 cas.

La fréquence de la maladie diminue ensuite brusquement, mais est encore notable jusqu'à 6 ans, pour tomber ensuite à des chiffres minimes pendant l'âge scolaire, l'adolescence et l'âge adulte.

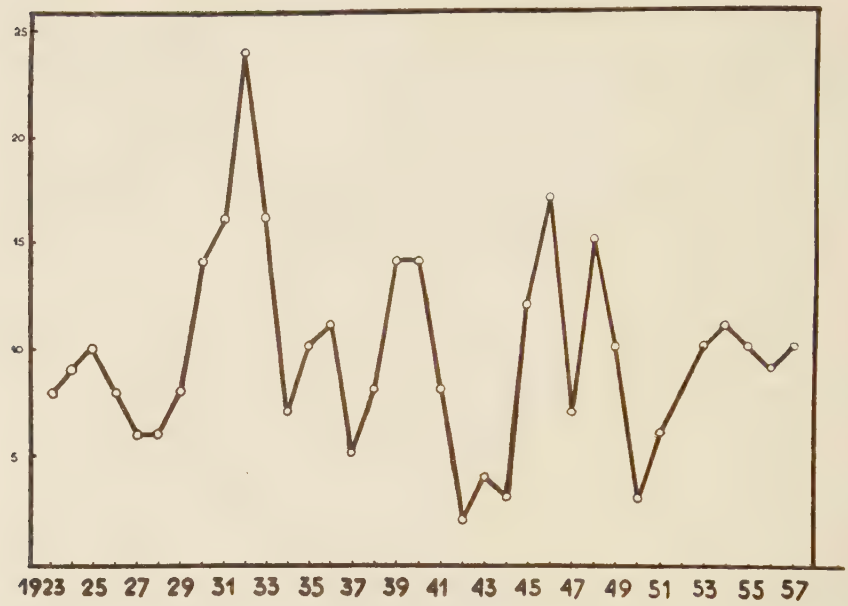



Fig. 1 — Nombre des cas de kala-azar de 1923 à 1957

Les chiffres suivants, qui donnent le total de nos 346 cas recueillis en 35 ans, illustrent bien cette notion, ainsi que le graphique correspondant (fig. 2):

| | |
|---|---|
| 0 à 1 an | 13 cas |
| 1 à 2 ans | 90 cas |
| 2 à 3 ans | 88 cas |
| 3 à 4 ans | 34 cas |
| 4 à 5 ans | 37 cas |
| 5 à 10 ans | 53 cas [1] |
| 10 à 15 ans | 12 cas [2] |
| 15 à 20 ans | 7 cas [3] |
| Au dessus de 20 ans et jusqu'à 56 ans | 12 cas |

[1] 10 cas par année environ.
[2] 2 cas par année environ.
[3] 1 cas par année environ.

Cette très nette prédominance de la maladie pendant le jeune âge est propre au kala-azar méditerranéen et nous en tirerons des déductions épidémiologiques importantes.

En particulier, nous ferons remarquer que c'est l'âge du contact avec le sol le plus intime et où l'enfant est exposé le plus fréquemment à la piqure des insectes courant sur le sol. Par contre, les insectes ailés piquent aussi facilement les nourrissons ou les grands enfants que ceux dont l'âge est compris entre 1 et 5 ans.

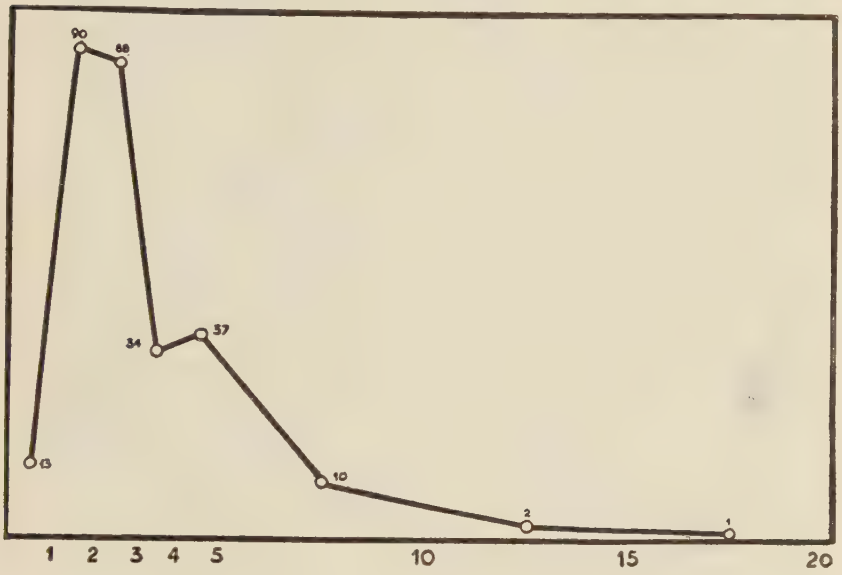



Fig. 2 — Fréquence du kala-azar suivant l'âge

## INFLUENCE SAISONNIÈRE

Il est très difficile de connaître le début réel d'une maladie dont les premiers symptômes sont peu révélateurs et qui est ensuite méconnue pendant des périodes parfois très longues.

Aussi le diagnostic du kala-azar infantile se fait-il en toute saison sans que l'on puisse en inférer la date réelle de l'infestation.

## RÉPARTITION GÉOGRAPHIQUE

Étudiant sur une carte de la région marseillaise la localisation des cas de leishmaniose, nous avons pu confirmer ce que d'autres auteurs avaient observé avant nous, c'est-à-dire la prédominance de

la maladie dans les quartiers périphériques et dans les zones sèches et broussailleuses plutôt que dans les régions humides.

Fig. 3 — Cas de leishmaniose humaine

Il y a des foyers de kala-azar très limités qui durent quelques années, puis qui cessent, et d'autres se déclarent dans des zones similaires, mais sans lien avec le précédent.

Nous pensons que la propagation à partir de chiens contagieux explique cette particularité.

En France, le domaine de la leishmaniose viscérale est très particulier, occupant à peu près exclusivement la côte méditerranéenne,

depuis le Rhône jusqu'en Italie et la rive droite du Rhône jusqu'aux environs de Lyon. Quelques cas aberrants ont été signalés ailleurs mais très peu nombreux.

Fig. 4 — Répartition géographique du kala-azar

Signalons, en particulier, que la rive gauche du Rhône est indemne, ainsi que la région marécageuse qui va du delta du Rhône jusqu'à la frontière espagnole.

Signalons, là encore, la prédominance de la maladie dans les régions sèches et l'absence paradoxale de cette même affection dans les régions humides, où les insectes ailés trouvent leur terrain d'élection.

## ENDÉMIE CANINE

Par son importance et sa chronologie, l'endémie de la leishmaniose canine mérite d'être mise au premier plan des études épidémiologiques concernant le kala-azar méditerranéen.

Il ne faut pas oublier, en effet, que Nicolle a découvert la leishmaniose canine à Tunis dès 1909, en même temps que la leishmaniose humaine.

Dans la région marseillaise, Pringault, élève de Nicolle, a signalé l'infestation des chiens dès 1914 et prédit dès cette date l'apparition de cas chez l'homme, alors que cette maladie humaine n'a été signalée qu'en 1922. Il estimait à cette époque que 2,18 % des chiens de notre ville étaient porteurs de parasites. Aussi, avons-nous pensé que l'étude de l'endémie canine marseillaise devait tenir une place importante dans nos travaux sur l'origine de la leishmaniose humaine dans notre région.

*Au point de vue clinique,* la maladie peut être à peu près complètement latente pendant de longues périodes. Nous avons relaté l'observation d'un enfant atteint de kala-azar qui avait comme commensal un petit fox paraissant en excellente condition générale. En raison de la maladie de l'enfant, le chien fut examiné attentivement et on ne trouva qu'une plaque de dépilation très limitée sur la région dorsale. Et, cependant, ce chien sacrifié fut reconnu porteur de leishmanias dans la peau et les organes internes.

Cette période de latence de la leishmaniose canine permet de supposer que la maladie est plus fréquente encore que l'on ne pourrait le croire d'après l'aspect extérieur des animaux et que seules des recherches sérologiques ou des prospections parasitologiques sont susceptibles de fixer le nombre relatif des animaux atteints de cette affection par rapport aux animaux sains.

Nous n'insisterons pas sur la double localisation simultanée de la leishmaniose, à la fois sur la peau et les organes internes. Cette double parasitation que nous pensons avoir été les premiers à signaler en 1932, au premier Congrès d'Hygiène Méditerranéenne, à Marseille, peut expliquer bien des points de l'épidémiologie et, en particulier, la redoutable contagiosité des chiens ainsi entourés d'un véritable manchon de leishmania.

Tout le monde connaît bien aussi les lésions viscérales de la maladie canine, portant surtout sur le foie, la rate et la moelle osseuse, mais diffusant aussi sur tout le système réticulo-endothélial.

Bien connus aussi sont les accidents cutanés à forme de dépilation et d'ulcérations, portant surtout sur la face, mais pouvant atteindre aussi les muqueuses oculaires et buccales.

Enfin, l'évolution chronique de l'affection entraîne à la longue des troubles généraux avec amaigrissement, des paralysies du train postérieur et enfin la mort par cachexie.

Mentionnons cependant une notion moins connue: la résistance toute particulière de la leishmaniose canine vis-à-vis de la thérapeutique spécifique par les sels d'antimoine. On obtient par ces médicaments des améliorations passagères à peu près toujours suivies de rechutes, à plus ou moins longue échéance. Peut-être ces insuccès sont-ils dus au retard souvent très grand de la thérapeutique et aussi au peu de continuité des traitements dominés par des considérations pécuniaires qui le font interrompre trop souvent avant la complète stérilisation de l'animal.

## PROCÉDÉS DE DIAGNOSTIC

Pour diagnostiquer la maladie canine, deux méthodes sont à mettre en parallèle: les examens sérologiques et les recherches parasitologiques.

Les *méthodes sérologiques* utilisent les mêmes procédés que pour la méthode humaine. Réactions de floculation à l'uréastibamine, au sulfarsénol, à l'eau distillée, au peptonate de fer, etc., ont été essayées. Dernièrement la réaction au thymol de Mac Lagan a été aussi utilisée avec des résultats intéressants. Mais il ne fait pas de doute que la formol-réaction ne soit de beaucoup la technique la plus facile à mettre en œuvre et la plus sure dans ses résultats.

Comme à toute méthode sérologique, on peut lui faire deux reproches opposés: d'abord, son manque de spécificité, car elle peut être positive en dehors de la leishmaniose dans un certain nombre de cas; ensuite, son manque de sensibilité, car elle n'est positive le plus souvent que dans les cas évoluant déjà depuis plusieurs mois au moins. Cependant, pour des dépistages sommaires et des statistiques d'infestation canine dans une région, elle peut être utilement mise en œuvre,

car le pourcentage des erreurs en plus ou en moins n'est pas considérable.

Les *recherches parasitologiques* sont certainement plus scientifiques et, en théorie, plus satisfaisantes parce que plus sensibles et donnant la certitude du diagnostic. Malheureusement, en technique vétérinaire, on se heurte à des difficultés pratiques qui en limitent l'emploi. La recherche des leishmanias est difficile dans la rate sur l'animal vivant, en raison de la forme allongée de l'organe, la ponction du foie et de la moelle osseuse est douloureuse et peu aisée à réaliser. Les frottis dermiques sont souvent positifs et de façon précoce, mais la recherche des leishmanias est souvent longue et fatigante par ce procédé.

Heureusement, nous disposons avec la ponction ganglionnaire d'une méthode pratique et sure qui nous a toujours donné d'excellents résultats. Dès 1936, nous avons signalé la facilité de cette ponction et sa fidélité pour le diagnostic parasitologique de la leishmaniose canine. Dernièrement, nous sommes revenus sur cette question avec de nouveaux documents confirmatifs. Sur 135 chiens suspects de l'affection, on a trouvé des leishmanias dans 98 cas. Nous signalons, en outre, que cette ponction est surtout positive au début, alors que les lésions cutanées sont discrètes ou absentes et la formol-réaction négative. Plus tard, les parasites deviennent plus rares, mais les lésions cutanées sont devenues caractéristiques et la formol-réaction positive. Enfin, dans les cas arrivés à un degré extrême de cachexie avec des lésions cutanées ulcérées et surinfectées, les parasites disparaissent du ganglion ou sont plus ou moins lysés et difficiles à caractériser. Cette méthode sera donc surtout précieuse pour le dépistage précoce de la maladie chez les animaux suspects et pour controler la guérison et dépister les rechutes après un traitement stibié.

## IMPORTANCE DE L'ENDÉMIE MARSEILLAISE

L'endémie canine, dans notre région, est d'une importance vraiment impressionnante, et voici quelques chiffres à l'appui de cette opinion.

Déjà Pringault, dans sa thèse, estimait à 2,18 % la proportion des chiens contaminés dans notre ville. Nous avons l'impression que

ces chiffres sont inférieurs à la réalité actuelle. L'un de nous, dans sa seule clientèle, en dépiste une trentaine de cas par an, et l'on peut estimer à une centaine environ ceux qui sont observés en tout annuellement dans notre ville. Si l'on réfléchit à ce fait que les chiens sont beaucoup moins nombreux que les hommes et que les chiffres qui les concernent sont dix fois plus forts environ, on voit que la leishmaniose canine est infiniment plus fréquente que le kala-azar humain, pour lequel il joue sans doute le rôle de réservoir de virus.

D'ailleurs, en France même, Faure-Brac, à Nice, estime à 18 % la proportion de chiens infestés, et Roubert, à Montpellier, note une proportion de 17 %. Sans doute, les chiffres en biologie doivent être considérés comme des indications plutôt que comme des valeurs absolues, mais ils situent tout de même la question.

Autre considération importante, la race des chiens ne paraît jouer aucun rôle sérieux et toutes les variétés sont mélées dans nos statistiques. Mais sur 100 cas dénombrés à ce point de vue nous notons:

51 chiens de chasse, soit 44 % environ;
43 chiens de garde, soit 37 % environ;
20 chiens d'agrément, soit 17 % environ.

Les chiens de chasse sont donc de beaucoup les plus touchés, puis viennent les chiens de garde, et nous verrons quelles conclusions épidémiologiques nous pouvons tirer de ces constatations.

Pour l'âge, c'est de 1 à 6 ans que les cas sont les plus fréquents. Notons que ce n'est pas une maladie des tout jeunes chiens.

## RÉPARTITION GÉOGRAPHIQUE

Si nous pointons sur une carte de notre région le domicile des propriétaires, nous trouvons une répartition qui ressemble de très près à la topographie des cas humains. Prédominance en banlieue et à la campagne, et, dans la ville, parasitation prédominante dans la région avoisinant la Corniche. Il y a cependant un plus grand nombre de cas urbains que pour le kala-azar humain, mais ces chiens domiciliés en ville sont souvent des chiens de chasse qui peuvent se contaminer au loin. Or nous avons vu la très grosse prédominance de ces chiens

dans notre statistique. Quant aux chiens de garde qui viennent aussitôt après, ils sont par définition des campagnards. Enfin, les chiens d'agrément, qui sont souvent élevés dans des appartements en

Fig. 5 — Cas de leishmaniose canine

ville, sont plus rarement atteints que les animaux des autres catégories.

Comme pour le kala-azar aussi, ce foyer d'endémie canine se relie à d'autres zones infectées dans tout le Sud-Est. À Nice, M. Faure-Brac a consacré à cette question des études très sérieuses et conclut, lui aussi, à la grande fréquence de l'affection. Toute la côte, depuis

Marseille jusqu'en Italie, est aussi contaminée. Quelques cas ont été signalés dans la région de Montpellier et en Ardèche.

Sur tout le pourtour de la Méditerranée, l'atteinte des chiens accompagne l'endémie humaine. Espagne, Italie, Grèce, Afrique du Nord, Asie Mineure sont le siège d'endémies canines importantes, et l'on ne peut voir là une simple coincidence. Par contre, dans l'Inde, les chiens seraient indemnes de la leishmaniose, alors que les hommes adultes, aussi bien que les enfants, sont fréquemment atteints par la maladie. Faut-il y voir une indication sur un mode de propagation particulier à cette région? Il n'est pas déraisonnable d'y penser.

## IDENTITÉ AVEC LE KALA-AZAR HUMAIN

Le parasite de la leishmaniose canine est-il identique à celui du kala-azar humain? Cette question ne peut être résolue de façon tout à fait formelle, mais des arguments très sérieux plaident pour une réponse affirmative.

Tout d'abord, morphologiquement et en culture, les deux variétés de leishmania sont absolument identiques. Ensuite, la coexistence des deux endémies humaine et canine dans tous les foyers méditerranéens et la répartition géographique identique dans l'intérieur de chaque foyer plaident dans le même sens.

De nombreux enfants atteints de kala-azar ont été en contact avec de chiens leishmaniées. D'ailleurs, Nicolle a découvert la parasitation des chiens par des recherches systématiques supposant *a priori* qu'ils pourraient être des réservoirs de virus et l'hypothèse s'est vérifiée exacte.

Enfin, il est possible d'inoculer la leishmania Donovani d'origine humaine au chien et Nicolle en a étudié l'évolution avec précision.

Sans doute, cette maladie expérimentale est-elle bénigne et spontanément curable, mais elle peut être réalisée à partir des formes de résistance seules observées dans l'organisme des animaux supérieurs.

On a fait de plus observer que la maladie du chien diffère notablement de la maladie humaine, en particulier par la présence de lésions cutanées constantes coexistant avec les lésions viscérales et par sa résistance beaucoup plus grande à la thérapeutique stibiée. Mais le terrain spécial sur lequel la maladie évolue peut expliquer

ces différences sans porter atteinte à la théorie de l'unicité des virus que nous considérons comme très probable.

1° Que la maladie est très fréquente, beaucoup plus fréquente que dans l'espèce humaine, de l'ordre sans doute de 15 à 18 % des chiens de notre région. Il s'agit donc d'une maladie habituelle du chien exceptionnellement transmise à l'homme;

2° Que chez l'animal la maladie évolue de façon torpide au début et souvent inapparente, pour ne se révéler que plus tard par des lésions cutanées caractéristiques qui accompagnent toujours la leishmaniose viscérale du chien;

3° Que le diagnostic est actuellement relativement facile par l'étude de la formol-réaction et la recherche des parasites par ponction ganglionnaire. L'une de ces méthodes complétant l'autre;

4° Que la répartition géographique identique de ces deux endémies sur tout le pourtour de la Méditerranée et l'identité probable des deux virus laissent supposer un lien direct entre les deux affections, soit que le chien contamine l'homme, soit que le chien et l'homme soient inoculés par un hôte intermédiaire.

## MÉCANISME DE TRANSMISSION DE LA MALADIE

Tout ce que nous avons observé nous porte à penser que la leishmaniose viscérale est, dans nos régions méditerranéennes, une maladie très fréquente chez le chien, cet animal jouant vis-à-vis de l'homme le rôle indubitable de réservoir de virus.

Nous sommes donc franchement uniciste et, de même que nous considérons que le parasite de la maladie hindoue est identique à celui de la forme méditerranéenne, de même nous pensons que le parasite de la maladie canine est identique à celui de la maladie humaine.

Les dénominations de *L. infantum* et *L. canis* nous paraissent devoir tomber en désuétude et seul le terme de *Leishmania donovani* doit être conservé.

Nous ne sommes d'ailleurs pas loin d'adopter le même point de vue pour *L. tropica*.

Seules des modalités, de climat, de terrain, d'agents vecteurs, déterminent la forme clinique et l'évolution de la maladie.

En partant de ces constatations initiales, pouvons-nous résoudre le problème de la transmission du kala-azar méditerranéen? Nous pensons que l'on peut l'affirmer avec une quasi certitude actuelle, mais nos conclusions à ce point de vue diffèrent sensiblement de celles des auteurs qui se sont occupés de cette question jusqu'à ce jour.

*La transmission directe de l'homme à l'homme* ne nous paraît guère à retenir.

Nous pensons que, contrairement à ce qui a été avancé par certains, il n'y a pas de leishmania au niveau de la peau dans le kala-azar méditerranéen.

Sans doute, le Prof. Fraga de Azevedo a signalé la présence de parasites dans le mucus nasal de ces malades et nous avons pu vérifier la véracité de ce fait. Mais nous n'avons, au cours d'une observation de 35 ans, jamais observé de contagion interhumaine certaine. De plus, nous avons relaté l'observation d'une famille où trois enfants ont été successivement atteints de leishmaniose viscérale. Mais ces cas familiaux sont survenus à un an au moins de distance les uns des autres, ce qui constitue un délai trop long pour admettre une transmission directe de la maladie.

Une possibilité a été cependant évoquée ces temps derniers à propos d'un cas singulier. Un malade atteint de leishmaniose cutanée aurait transmis le kala-azar par transfusion sanguine à une enfant (Brumpt). Cette modalité serait évidemment possible à imaginer, mais il est peu probable qu'un sujet atteint de kala-azar soit utilisé comme donneur de sang et il s'agit là d'une simple curiosité exceptionnelle qui ne sera jamais à retenir pour la transmission habituelle de cette affection.

*La transmission directe du chien à l'homme* serait plus facile à imaginer, puisque chez cet animal les téguments sont parasités et il n'est pas impossible de penser que la contamination puisse se faire ainsi à l'occasion de petites effractions cutanées. Il est possible, en effet, de transmettre la maladie aux animaux d'expérience à partir de formes de résistance sans recourir au passage par un insecte ou arthropode vecteur qui seul permet le développement des formes flagellées du parasite.

Cependant, là encore, les observations cliniques ne sont guère en faveur de ce mécanisme et un nombre suffisant d'observations d'enfants ayant contracté le kala-azar sans avoir été en contact direct avec des chiens a été publié dans divers pays pour que l'on puisse affirmer que ce n'est pas ainsi que la maladie se transmet de façon habituelle.

Force est donc pour nous, comme pour tous les auteurs qui se sont occupés de cette question, d'admettre la nécessité de l'intervention d'un insecte ou arthropode vecteur.

On a incriminé autrefois la punaise, les puces, le pou du chien *(Cignonathus piliferus)*, mais il ne semble pas que le rôle de ces animaux qui peuvent cependant présenter des protozoaires flagellés dans leur tube digestif soit vraisemblable en pratique.

Les *phlébotomes* ont été considérés comme les vecteurs probables de la maladie à la suite d'expériences de transmission de la leishmaniose viscérale aux animaux de laboratoire, quoique, à dire vrai, ces expériences aient été très rarement suivies de succès. Ces mêmes phlébotomes sont aussi considérés comme les agents vecteurs de la leishmaniose cutanée (bouton d'orient).

Ils sont abondants dans notre région et piquent aussi bien les animaux que l'homme, et, de ce fait, leur rôle paraît possible dans la transmission du kala-azar méditerranéen. Cependant, nous ferons remarquer que le chien n'est pas l'animal de choix pour eux et on s'expliquerait difficilement la très grande fréquence de la leishmaniose canine qui caractérise notre région.

De plus, la répartition géographique de la maladie en France ne coïncide pas du tout avec la répartition des phlébotomes. De vastes régions où ces insectes sont abondants, comme le sud-ouest de notre pays, sont indemnes de leishmaniose canine et humaine. Le caractère local des épidémies persistant des années dans un même territoire très restreint (maisons à kala-azar) et attaquant à plusieurs années de distance les membres d'une même famille, ne franchissant pas une barrière fluviale comme le Rhône, ne paraît pas non plus en faveur du rôle d'un insecte ailé dont l'aire de diffusion est toujours plus étendue et moins précise.

Enfin, chez l'homme lui-même la prédominance singulière et frappante de la maladie chez les enfants de 1 à 3 ans ne s'explique pas aisément si l'on retient le rôle du phlébotome. Ceux-ci piquent

en effet aussi bien des enfants pendant leur première année et ceux qui sont âgés de 3 à 6 ans.

*Nous dirons donc,* avec le Prof. Fraga de Azevedo, *que l'épidémiologie de la leishmaniose viscérale dans notre région méditerranéenne ne peut pas s'expliquer si l'on pense que le phlébotome est l'agent vecteur de la maladie. Au nom des faits que nous avons observé avec netteté et certitude, nous pensons pouvoir rejeter ce mécanisme de transmission en ce qui concerne le kala-azar méditerranéen.*

*Le rôle de la tique* a été soupçonné par Nicolle, et ultérieurement Blanc et Caminopetros ont réussi l'infestation du hamster à partir du broyat de tiques infectées. Ils ont montré aussi que le parasite persistait dans l'organisme de la tique après sa métamorphose de nymphe en adulte parfait. Malheureusement, on n'a jamais pu faire infecter des animaux par piqûre directe de ces tiques et la preuve formelle de leur rôle nous échappe ainsi encore.

Cependant, au point de vue épidémiologique, dans la région méditerranéenne nous pouvons donner les arguments suivants qui nous paraissent d'une force singulière.

D'abord la tique est un parasite habituel du chien. Dans notre région, pas un seul chien n'échappe à la piqûre de cet arthropode, et il faut débarrasser tous les jours les chiens de ces hôtes incommodes et dangereux pendant la saison chaude, et surtout au printemps (adultes parfaits) et en automne (larves et nymphes). Ces tiques piquent aussi l'homme de façon non exceptionnelle, et la transmission de la fièvre boutonneuse méditerranéenne est due à cette piqûre des tiques. Enfants et adultes sont ainsi inoculés, mais bien entendu moins souvent et moins régulièrement que les chiens. Ainsi, par cette hypothèse se trouveraient expliquées la considérable parasitation des chiens et la plus rare infection de l'homme. La tique incriminée dans notre région est *Rhipicephalus sanguineus.*

Ensuite on explique aussi de cette manière la prédominance singulière de la maladie chez les enfants de 1 à 4 ans, qui est une des caractéristiques du kala-azar méditerranéen. En effet, les nourrissons relativement à l'abri dans leur berceau sont rarement piqués par les tiques; les grands enfants et les adultes se défendent aussi mieux contre leurs piqûres. Par contre, les enfants de 1 à 4 ans jouent sans défense sur le sol ou dans les buissons où se trouvent les tiques aux

aguets cherchant une proie pour faire le repas de sang nécessaire à chacune de leurs transformations.

De plus, l'étude de la répartition géographique de la leishmaniose canine et humaine, qui est à peu près identique, fait ressortir la prédominance de la maladie en banlieue ou dans les zones où se trouvent de grands jardins. Tous les auteurs qui se sont occupés du kala-azar méditerranéen ont noté la chose comme nous.

Dans notre ville aussi, nous retrouvons une zone de forte endémie dans un secteur où des collines broussailleuses et des pinèdes se trouvent enclavées en plein cœur de l'agglomération urbaine. Or, les tiques pullulent dans ces zones sèches et rocailleuses couvertes d'arbustes épineux et de pins. Elles se logent pendant l'hiver dans les écorces des arbres, dans les fissures des murs, dans les anfractuosités des rocailles à l'abri du vent et souvent ensoleillées. L'été, elles sortent de leurs repaires et courent sur le sol, grimpent sur les buissons et attendent leur proie ou vont à la découverte. On voit ainsi parfois des files de ces arthropodes courir sur les murs et le sol dans la saison favorable.

Ainsi le fait d'admettre le rôle des tiques dans la transmission de la leishmaniose permet d'expliquer de façon raisonnable les trois grandes caractéristiques de l'endémie méditerranéenne:

Infestation extraordinairement fréquente des chiens et infestation plus modérée de l'espèce humaine;

Prédominance du kala-azar méditerranéen chez les enfants de 1 à 4 ans;

Répartition géographique des deux endémies qui prédominent nettement en banlieue et à la campagne, là où se trouvent des collines sèches et broussailleuses couvertes de pinèdes.

Ces constatations épidémiologiques viennent renforcer les résultats expérimentaux malheureusement incomplets observés par Blanc et Caminopetros et leur donnent un relief tel qu'il n'est pas possible de les tenir pour négligeables.

Il n'est pas interdit de penser d'ailleurs que les diverses variétés de leishmaniose humaine seraient dues à des modes d'inoculation différents suivant les régions.

Les phlébotomes pourraient aussi être les agents de transmission du kala-azar indien et aussi des leishmanioses cutanées et les tiques l'arthropode vecteur du kala-azar méditerranéen et de la leishmaniose canine.

Cette conception uniciste semble confirmée par une observation récente de Mrs. André et Brumpt concernant un sujet atteint de leishmaniose cutanée après piqûres de phlébotomes qui donna par transfusions sanguines une leishmaniose viscérale à un enfant. Mais cette observation unique ne peut sans doute être considérée comme une preuve définitive de notre conception.

Cependant, il y a là plus que des hypothèses et les preuves épidémiologiques que nous donnons nous semblent convaincantes et, en tout cas, de nature à faire réviser nos idées sur la transmission du kala-azar méditerranéen.

## PROPHYLAXIE

Les considérations concernant l'épidémiologie du kala-azar méditerranéen rendent facile l'étude des procédés prophylactiques à mettre en œuvre pour éviter cette maladie.

*Le chien* étant dans nos régions le principal réservoir de virus, nous devons préconiser l'abattage systématique des chiens errants, la surveillance régulière de tous les chiens pour dépister la leishmaniose, soit par la formol-réaction, soit par la mise en évidence du parasite par ponction ganglionnaire.

Tout chien reconnu atteint de cette affection devrait être abattu en raison de la difficulté du traitement stibié chez cet animal et l'incurabilité habituelle de la maladie.

Enfin, tous les chiens quels qu'ils soient doivent être déparasités de leurs tiques quotidiennement pendant la saison favorable de mai à octobre.

Tout ceci étant difficile à réaliser en pratique, il serait mieux encore d'écarter systématiquement les chiens du voisinage des enfants. On les préservera ainsi à peu près surement de deux grandes parasitoses: le kyste hydatique et le kala-azar.

Les *insectes ou arthropodes vecteurs,* tiques et phlébotomes, doivent aussi être détruits systématiquement dans toutes les régions suspectes à ce point de vue.

Il est en effet difficile de préserver les enfants de la piqûre des phlébotomes, qui passent même à travers les mailles des moustiquaires. Il est difficile aussi d'empêcher les enfants de 1 à 4 ans de se trainer sur le sol.

D'aileurs, dans certaines régions d'Italie en particulier, où le paludisme sévissait autrefois de façon sévère, on a vu l'endémie de la leishmaniose diminuer jusqu'à presque disparaître sous l'influence d'une campagne de désinsectisation dirigée contre l'endémie palustre.

Peut-être est-ce en raison de l'absence de cette lutte contre les insectes que la région méditerranéenne française, qui est indemne d'infestation palustre, voit se perpétuer une endémie de kala-azar qui est parfaitement stable depuis de longues années.

Il est donc à recommander d'entamer dans nos régions une lutte contre les parasites piqueurs (phlébotomes, moustiques, tiques), soit avec le classique DDT, soit avec les parasiticides plus puissants (HCH).

Il est à noter cependant que les tiques plus résistants que le phlébotome, se réfugiant dans des repaires peu accessibles (écorce des arbres, creux des murailles, fissures des parois rocailleuses) et sur le sol et dans les buissons épineux, sont beaucoup moins faciles à atteindre que les insectes ailés, ce qui expliquerait ainsi la relative résistance de l'endémie aux campagnes de désinsectisation.

Ce double aspect de la prophylaxie du kala-azar méditerranéen (lutte contre la leishmaniose canine et campagne de désinsectisation) nous paraît résumer à l'heure actuelle les mesures de protection vis-à-vis d'une maladie vraiment non négligeable dans le monde et tout particulièrement dans notre pays, où les autres parasitoses ont été très efficacement neutralisées.

Il ne faut pas oublier, en effet, que le paludisme a sévi autrefois dans plusieurs régions de France (Alsace, Vendée, Pyrénées Orientales, Camargue), mais a pratiquement disparu de notre pathologie autochtone.

Il faut souhaiter qu'il en soit de même de la leishmaniose sous toutes ses formes et tout particulièrement du kala-azar infantile, qui n'aboutit actuellement plus à la mort des malades, mais qui réalise des affections graves et souvent de longue durée.

## CONCLUSIONS

Nous dirons en conclusion:

Que l'endémie de la leishmaniose humaine méditerranéenne étudiée par nous depuis 35 ans à propos du foyer marseillais est une endémie stable et relativement importante (10 cas par an en moyenne à la Clinique Médicale Infantile);

Qu'elle coexiste avec une endémie canine plus importante encore qui a paru la précéder dans le temps et l'accompagne de façon très étroite. Le chien paraît donc être le réservoir de virus majeur de la maladie;

Que la maladie ne paraît pas se transmettre directement du chien à l'homme, ni de l'homme à l'homme, et qu'il est vraisemblable que cette transmission se fait habituellement par l'intermédiaire d'un animal vecteur (insecte ou arthropode);

Que parmi ces vecteurs possibles le phlébotome à été désigné par des expériences de laboratoire par ailleurs critiquables, mais que cette hypothèse ne s'accorde nullement avec ce que nous savons de l'épidémiologie du kala-azar méditerranéen;

Que par contre, si le rôle des tiques n'a pas été absolument démontré, il existe cependant des expériences de Blanc et Caminopetros qui permettent le doute au sujet de ce mécanisme de transmission. Par ailleurs, toutes les données de l'épidémiologie sont en faveur de cette intervention des tiques;

Que la prophylaxie actuelle de la maladie paraît devoir se résumer en deux directives essentielles:

*Faire disparaître l'endémie canine* par l'abattage des chiens errants et des animaux reconnus malades.

*Lutter contre les insectes ou arthropodes* suceptibles de servir d'hôtes intermédiaires, tiques et phlébotomes, en entreprenant des campagnes type campagnes antipaludiques, pour détruire ces animaux vecteurs avec les moyens les plus puissants dont nous disposons.

Le succès de telles campagnes dans certaines régions de la zone méditerranéenne est encourageant à ce point de vue.

## BIBLIOGRAPHIE

Nous nous excusons du caractère incomplet de cette bibliographie, qui se borne à quelques références importantes sur la question.


ADLER — «La leishmaniose viscérale canine et ses relations avec la leishmaniose viscérale humaine». *III° Congr. Intern. de Patho. comparée,* février, 1937.

BERGIER — *Épidémiologie de la leishmaniose viscérale dans la région marseillaise.* Thèse de Marseille, 1939.

BLANC et CAMINOPETROS — «Quelques expériences sur la transmission du kala-azar par la tique du chien», *C. R. Soc. Biol.,* 18 sept., 1931.

CABASSU — *Leishmaniose spontanée du chien.* Thèse vétérinaire, Paris, 1933.

CORDOLIANI — *Épidémiologie de la leishmaniose viscérale en Corse.* Thèse de Marseille, 1940.

DONATIEN et LESTOQUARD — *Leishmanioses animales,* Soc. de Path. comparée, Janvier, 1936.

FAURE-BRAC — *La leishmaniose canine.* Thèse vétérinaire, Lyon, 1933.

FRAGA DE AZEVEDO — «L'état actuel du problème du kala-azar au Portugal», *Arch. Institut Pasteur d'Algérie,* t. XXXII, n° 3, sept., 1954.

FRAGA DE AZEVEDO — «Sur la transmission du kala-azar méditerranéen», *Anais do Instituto de Medicina Tropical,* vol. V, décembre, 1948.

GIRAUD, P., et CABASSU — «Sur la présence des parasites dans les lésions cutanées muqueuses du chien atteint de leishmaniose spontanée», *Congr. Intern. d'Hyg. Méd.,* Marseille, 1932.

GIRAUD, P., et CABASSU — «Sur la valeur des procédés de laboratoire pour le diagnostic de la leishmaniose canine naturelle», *Annal. de l'Institut Pasteur,* avril, 1933.

GIRAUD, P., et CABASSU — *Le diagnostic de la leishmaniose canine par la ponction ganglionnaire,* Soc. de Pathol. Exotique, novembre, 1936.

JOAQUIM EDUARDO DE ALENCAR — «Leishmaniose visceral no novo mundo», *Publica Medica Annal.,* XXVII, n.° 196, 1956.

NICOLLE et COMPTE — «Origine canine du kala-azar», *Arch. Institut Pasteur,* Tunis, 1908.

PRINGAULT — *Recherches sur la leishmaniose canine.* Thèse de Montpellier, 1917.

RANQUE, J., et CABASSU — *Le diagnostic précoce de la leishmaniose canine par la ponction ganglionnaire,* Acad. de Méd., 25 mars, 1948.

ROUBERT — *Recherches sur la leishmaniose canine dans la région de Montpellier.* Thèse de Montpellier, 1936.

POUL — «Sur la fréquence de la leishmaniose canine à Alger et sur la valeur diagnostique de la formol-gélification», *Arc. de l'Institut Pasteur d'Algérie,* t. XXVIII, n° 4, p. 449, 1950.

M.me BENREKASSA-PROVOST — *Épidémiologie du kala-azar dans le bassin méditerranéen français.* Thèse de Marseille, 1954.

ANDRE, R., BRUMPT, L., et coll. — *Leishmaniose cutanée, leishmaniose cutanéo-ganglionnaire et kala-azar transfusionnel,* Soc. Méd. Hôp. Paris, 18 octobre, 1957.

CAYLA, A., PERROT, A., et coll. — «Sur un cas de kala-azar transfusionnel chez un nourrisson de huit mois», *Arch. Fr. Péd.,* **14** (7), 1957.

MARGAROT, J., RIMBAUD, P., et coll. — «Deux nouveaux cas de bouton d'orient autochtones», *Montpellier Médical,* **44**: 34, 1953.

SAN GUPTA et DAS GUPTA — «Cutaneous lesions following inoculation of live culture of *Leishmania donovani* in man», *Indian Med. Gaz.,* **86** (1-6), 1951.

# SOME ASPECTS OF RESEARCH ON *LEISHMANIA TROPICA*

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Oriental sore lends itself readily to clinical investigation, but it is not necessary at this stage to summarize the extensive literature dealing with various clinical manifestations of the disease. Diagnosis by direct smears, culture and specific skin tests is not difficult and a fairly detailed knowledge of the epidemiology of most foci of the disease is therefore available. Difficulties in diagnosis are liable to arise only in cases of recurrent cutaneous leishmaniasis, some of which resemble lupus clinically. Parasites are extremely rare in these cases, so much so that they are only rarely seen in smears, but the *Leishmania* test is a help in diagnosis which can generally be clinched by a positive culture.

There are still lacunae in knowledge of the epidemiology of the disease in French West Africa and Western Nigeria, from which Jeliffe has reported a number of cases.

The known foci which have been sufficiently investigated are of three types:

1) Few or none escape the disease and dogs are frequently infected, e.g. Baghdad;
2) Human cases are fairly common though a larger part of the population is not infected. Infections in dogs have not been recorded, e.g. Israel;
3) Rural areas where the disease is maintained as a epizootic in gerbils and man is an accidental host, e.g. Turkestan.

The characteristics of each type of focus is determined by the prevalence and bionomics of the local sandfly vector, e.g. *Phlebotomus sergenti,* the main carrier in Baghdad, *P. papatasii,* in Israel, *P. papatasii* and *P. caucasicus,* which Russian authors have shown to be the vectors of *L. tropica* to gerbils in whose burrows these sandflies breed.

Although the transmission of *L. tropica* by the bite of sandflies has been definitely proved (1941), many details still remain to be investigated. *Leishmania tropica* is not a homogeneous species and there are distinct biological races which differ in their adaptation to various species of *Phlebotomus.* Thus *L. tropica* from Crete, unlike the Jericho strain, does not produce high infection rates in *P. papatasii.*

In Crete *P. sergenti* is the carrier and *P. papatasii* is of no importance as a vector. A very striking example of biological differences between two strains of *L. tropica* was demonstrated by Russian authors (Latischev & Krinkova, 1941) in the case of the rural and urban cutaneous leishmaniases of Turkestan. The two types are clinically distinct and it was demonstrated experimentally that there is no cross immunity between them. It should be stressed that, although the existence of distinct biological races of *L. tropica* has been demonstrated, no serious attempt has as yet been made to compare their antigenic structure. Because of clinical similarity between Old World and New World cutaneous leishmaniasis, one might expect a closer antigenic affinity between *L. tropica* and *L. brasiliensis* than between *L. tropica* and one of the visceral leishmanias. Curiously enough, this is not the case; comparing *L. tropica, L. brasiliensis, L. infantum* with a strain of a *Leishmania* sp. isolated by Dr. R. B. Heisch from a lizard *Latastia caudata* in Kenya, it was found in cross agglutination experiments that the specific serum of the lizard *Leishmania* produced high titres (up to 1 in 809) with *L. infantum* and *L. brasiliensis* and very low titres (1 in 50) with *L. tropica. Leishmania tropica* is also unique in its nutritional requirements among the known leishmanias of mammals and lizards. Thus Citri & Grossowicz (1955) have elaborated a partially defined culture medium (with albumen as the only constituent derived from serum and all other constituents chemically defined) on which *L. tropica* produces rich cultures, but which does not support the growth of *L. brasiliensis* or other mammalian leishmanias.

As previously stated, the transmission of *L. tropica* by the bite of *P. papatasii* has been proved (Adler & Ber, 1941) and the life cycle of *L. tropica* in the above sandfly is known in considerable detail (Adler & Theodor, 1926; Parist & Donuctien, 1927), but more research is required in this field. *Leishmania* species in nature are attuned to the physiology of their respective specific vectors. Specificity is manifested by high infection rates and heavy infections in the vectors following the ingestion of relatively few parasites together with a definite pattern of behaviour which includes multiplication in the midgut and a progression of the flagellates towards the biting parts. Sandflies transmit by bite only if the proboscis is invaded by flagellates and this invasion of the proboscis is determined by factors in the vector which may not be identical in different vectors. It is therefore desirable to investigate the details of transmission in different endemic foci particularly so as there are distinct biological races of *L. tropica* and differences in the bionomics and physiology of the various vectors. The vector of the disease in West Africa still remains to be determined.

It is generally conceded that for any single endemic focus one attack produces immunity. Marzinowsky & Schurenkova (1924), on the basis of a number of experiments on volunteers, concluded that complete immunity occurs only in those cases which are allowed to develop and heal naturally. These authors proved that if experimental lesions are removed surgically in their early stages and not allowed to run their natural course, no immunity is established and the volunteers can be re-infected.

Dostrovsky *et al.* (1952), in an interesting series of experiments, showed that in the case of relapsing cutaneous leishmaniasis which has a very definite clinical and histopathological picture, superinfection produces lesions very similar to the concurrent ones. The series of local cellular changes throughout the course of the infection are typical. There is in the first place a proliferation of reticulum cells in which the parasites multiply. This is eventually followed by a secondary infiltration of round cells and plasma cells associated with a fall in the number of parasites in the reticulum cells. The association of this secondary infiltration with a diminution in the number of parasites is of special interest in view of modern theory that plasma cells liberate antibodies. The immunity following natural cure is solid,

generalised for the whole dermis and is probably associated with humoral factors which have not yet been demonstrated *in vitro*. It has already been pointed out (Adler & Zuckerman, Dostrovsky *et al.*) that the antibodies involved in the specific skin reaction which is so useful in diagnosis have no protective value, for they are present in the early stages of the disease when multiplication of parasites is proceeding. It should be pointed out that not all parasites injected into the skin of an immune subject are destroyed rapidly, for cultures made after 24 hours from the site of inoculation may be positive.

The above brief survey clearly indicates that, in spite of decades of research on cutaneous leishmaniasis by numerous workers, there is still ample scope for further work.

## BIBLIOGRAPHY

Adler, S., & Theodor, O. — *Ann. Trop. Med. & Parasitol.*, **20**: 355, 1926.

Adler, S., & Ber, M. — *Ind. J. Med.*, **4**: 803, 1941.

Citri, N., & Grossowicz, N. — *J. Gen. Microbiol.*, **13**: 273, 1955.

Dostrovsky, A., Sagher, F., and Zuckerman, A. — *A. M. A. Arch. Dermat.*, **60**: 65, 1952.

Marzinowsky, E. J., & Schurenkova, A. — *Trans. R. Soc. Hyg. & Trop. Med.*, **17**: 67, 1924.

# LA LEISHMANIOSE EN ITALIE

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Notre intention a été de mettre en relief, dans une assez ample relation dont nous présentons ici un simple résumé, les études accomplies en Italie sur la leishmaniose depuis la description des premiers cas des différentes formes de cette parasitose, sur la situation épidémiologique de la maladie et sur tous les problèmes étiologiques, cliniques, hygiéniques, prophylactiques et thérapeutiques qui sont particuliers à notre pays.

Devant l'ampleur des données historiques et de la bibliographie sur ce sujet, nous avons eu l'avantage de pouvoir compter sur la relation que notre Maître, Mr. le Prof. Mario Girolami, présenta en 1948 au 6ème Congrès Italien de Médecine Tropicale et Subtropicale sur la «Leishmaniose viscérale en Italie» et sur celle de Mr. le Prof. F. Pullé présentée au même Congrès sur la «Leishmaniose cutanée».

Nous avons aussi eu l'avantage de jouir de notre expérience personnelle et de celle de l'École de Médecine Tropicale de Rome, qui fut jadis dirigée par Son Excellence le Prof. Aldo Castellani et qui est actuellement sous le guide de Mr. le Prof. Mario Girolami.

Après avoir rappelé que la leishmaniose est une maladie protozoaire qui se présente sous trois formes bien différentes (viscérale, cutanée, cutanéo-muqueuse) et après avoir rappelé que la forme viscérale était connue en Italie par T. Cigliano (1876) et par A. Cardarelli (1880), que le mérite d'avoir observé le parasite le premier appartient a Pianese (1905), tandis que les premiers cas de bouton

d'orient ont été décrits par Gabbi e La Cava (1910), nous avons examiné toutes les questions relatives à l'identité des différents parasites qui ont intéressé un grand nombre d'auteurs (Pianese, Gabbi, Giugni, Laurinsich, Bianchi, etc.).

Les problèmes des cultures ont été aussi largement étudiés en Italie (Caronia, Archetti, Zambrano, Biava, etc.), comme d'ailleurs les phénomènes immunitaires (agglutinations spécifiques, réaction de Bordet et Gengou, les anticorps allergiques, etc.).

Nous avons rappelé les associations morbides (avec fièvre paludéenne, tuberculose pulmonaire, fièvre de Malte, amibiase, etc.). À ce propos de nombreuses études ont été faites dans la Clinique de Rome.

Nous rappelons à peine les études sur la symptomatologie des différentes formes de leishmaniose auxquelles les auteurs italiens se sont particulièrement dédiés: comme à celles des relations entre leishmaniose et système réticulo-istyocitaire, à celles des réservoirs de virus, des vecteurs de virus, etc.

Dans notre relation nous nous sommes occupés assez largement des conditions relatives à l'épidémiologie de cette maladie en Italie.

La déclaration des cas de leishmaniose viscérale est obligatoire en Italie depuis 1927. En 1940 la déclaration aux autorités sanitaires fut rendue obligatoire pour les deux formes, cutanée et viscérale, et en 1946 elle fut rendue obligatoire pour les deux formes séparément.

Actuellement nos statistiques officielles s'arrêtent à 1954; les *Bulletins de l'Institut Central de Statistique* des années suivantes sont sous presse.

En examinant la courbe des cas de leishmaniose viscérale entre 1930 et 1954 on peut noter que des 264 cas de 1930 on arrive aux 48 cas de 1954, en passant par une pointe de 337 cas en 1938 et par une de 583 cas de 1946.

Pour les cas de leishmaniose cutanée, en examinant la courbe entre 1946 et 1954 on passe des 291 cas de 1946 aux 2.742 de 1948 et on descend jusq'aux 491 cas de 1954.

Pour ce qui concerne la distribution par région, on peut remarquer que la maladie est surtout diffusée dans les régions méridionales et dans les îles: Calabre, Pouilles, Lucania, Campanie, Sicile, Sardaigne. Les deux foyers les plus importants sont aux alentours des plus importants volcans (Naples et Catane).

La leishmaniose cutanée est plus particulièrement fréquente dans les Abruces, les Marques, la Romagne, la Calabre.

En examinant les rapports de distribution entre la forme cutanée et la forme viscérale on peut noter (Bevere et Tobia) que dans certaines régions les cas de leishmaniose viscérale sont prévalents, que dans d'autres ce sont les cas de leishmaniose cutanée qui prévalent et que dans d'autres encore les deux maladies sont à peu près au même niveau.

Il faut donc conclure que pour les régions italiennes on ne peut démontrer une constante corrélation ni un antagonisme entre les deux formes.

Pour la fréquence des deux maladies selon la saison, le maximum de cas a été déclaré au mois de mai.

Pour le sexe et l'âge, la leishmaniose viscérale est beaucoup plus fréquente au dessous des 3 ans, avec le maximum à 2 ans; elle diminue de 3 à 5 pour augmenter encore de 5 à 9 et pour diminuer très rapidement de 15 à 60 ans.

Le sexe masculin est, en valeurs absolues, le plus touché.

Pour la leishmaniose cutanée le maximum de fréquence est entre 5 et 25 ans: il n'y a pas de grande différence pour le sexe jusqu'à l'âge de 30 ans; plus tard le sexe féminin est un peu plus touché

Quelques considérations peuvent être faites pour les localités les plus touchées par les deux maladies. Nous avons déjà nommé les régions qui ont le plus grand nombre de cas. En général les communes où les maladies sont plus fréquentes sont situées de préférence le long des côtes et des fleuves.

Pour les autres considérations nous renvoyons à notre relation, dans lequelle nous avons aussi examiné les traitements des différentes formes depuis le traitement stibié employé dès 1914 par Di Cristina et Caronia en Italie et par Castellani à Ceylon dans la leishmaniose viscérale et le traitement à l'atebrine employé par Flarer dans la leishmaniose cutanée jusqu'aux traitements employés de nos jours.

# RECHERCHES SUR LA LEISHMANIOSE EN BULGARIE ET LES MÉTHODES DE SON DIAGNOSTIC

P. PAVLOV
Sofia, Bulgarie

Les conditions géoclimatiques dans notre pays sont favorables à l'existence de maladies exotiques. Les recherches faites jusqu'à présent montrent que certaines de ces maladies existent en quantité non négligeable. Pavlov et Guénev (1939 et 1941) ont constaté la présence de *Trypanosoma evansi*. Pavlov (1940 et 1942) a constaté aussi la maladie dénomée «Tick paralysis». De plus, il a constaté la présence dans le pays des tiques *Ornithodorus lahorensis* et *Amblyomma hebraeum*. Les recherches sur les tiques on montré la présence dans le pays de plusieurs espèces de tiques — jusqu'à présent 22 espèces, quelques unes de ces espèces sont réservoirs de protozoaires, de *Rickettsia* et probablement de virus. Les recherches sur la présence de virus chez les tiques sont en cours pour le moment.

Parmi les maladies exotiques du pays on a constaté aussi la présence de leishmaniose chez l'homme, chez le chien et dérnièrement chez le *Citellus citellus* par Pavlov. Les recherches faites de Pavlov (1943-1957) ont montré la présence du parasite chez le chien et chez le *Citellus citellus* comme réservoir du parasite dans la nature. Juroukov a constaté le premier cas autochtone de la maladie dans la région de Ajtos. Doganov (1945), Grouev et Janev (1949) ont constaté la maladie chez l'homme. La maladie est constatée chez l'homme aussi de Jonkov, 3 cas, et de Br. Bratanov (1952) dans 40 cas chez des enfants. C'est Pavlov qui a isolé pour la première fois le parasite du chien indigène en 1943.

Dans le présent travail nous exposons nos études sur la leishmaniose, ayant en vue l'importance de la maladie au point de vue

hygiénique, fait établi encore en 1908 par Charles Nicolle et ses successeurs. Tenant compte de la situation exposée, dans le pays nous avons entrepris de recherches sur la présence de la maladie chez le chien, comme hôte principal dans la nature, sur les méthodes les plus sûres pour un diagnostic exacte, ainsi que sur la présence du parasite dans l'organisme des animaux sauvages, comme réservoirs de cette maladie dans la nature.

Dans nos recherches tous les 120 chiens, soumis à nos expériences, ont été examinés par la réaction de Napier. Les chiens, ayant donné un résultat positif, ont été examinés cliniquement, en vue de savoir si, parmi eux, il y a des chiens qui montrent des symptômes cliniques de la maladie. Après cet examen, les chiens qu'ont réagi sérologiquement (après 30 minutes, 1-24 heures) ont été sacrifiés pour rechercher des lésions anatomo-pathologiques. De plus, on a fait des frottis, des ensemencements sur N.N.N. et recherches histologiques de la rate, du foie et de la moelle osseuse. Ces recherches faites, on a comparé les résultats, pour connaître la méthode la plus sûre de diagnostic.

Les résultats de ces recherches montrent que de 120 chiens examinés et provenant de différentes parties du pays 48 (40 %) ont donné un résultat positif à la recherche sérologique. De ces 48 chiens qui ont réagit positivement à la recherche sérologique seulement 4 (10 %) ont montré des ulcérations sur le museau, leur poils étaient tombés autour des yeux et ces dérniers étaient inflamés.

Les recherches microscopiques, histologiques et sur N.N.N. ont donné les résultats suivants:

De 48 chiens examinés par la méthode microscopique sur les frottis de moelle osseuse, de la rate et du foie (10 frottis pour chaque organe et par chien), colorés au Giemsa, nous avons constaté la présence du parasite chez 12 chiens (25 %). Le parasite est constaté, chez tous les chiens, dans la moelle osseuse et, chez 10 chiens, dans les frottis du foie et de la rate.

Le résultat était le même dans la recherche histologique.

Ce résultat montre que la recherche histologique n'a aucune supériorité sur la recherche microscopique des frottis de la moelle osseuse, du foie et de la rate. De plus, la méthode de recherche sur des frottis est plus facile et plus simple.

La recherche faite d'après l'ensemencement sur le milieu N.N.N. et les cultures obtenues a donné un résultat positif chez 10 chiens (20 %).

Le résultat de nos recherches montre que la maladie existe dans notre pays. Une lutte organisée sur une base scientifique est également entreprise en même temps avec la lutte contre la rage. Cette lutte a limité la quantité des chiens dans la pays, 5 fois, après l'année 1944 — l'année pendant laquelle a commencé la coopération de la terre dans nos villages.

La méthode la plus sûre de diagnostic de la maladie est la recherche microscopique sur les frottis de la moelle osseuse, de la rate et du foie, ainsi que la culture sur le milieu N.N.N. Cette dernière méthode doit être pratiquée après un résultat microscopique négatif.

Le résultat exposé montre qu'il n'existe pas de relation entre l'apparition du gel et la maladie du chien. Il y a des chiens indemnes qui ont montré une réaction sérologique de Napier positive.

On a constaté que le parasite a des réservoirs dans la nature en Bulgarie, parmi les animaux sauvages. Des recherches encore plus détaillées sont en cours sur cette question. D'après Pavlovski, c'est une maladie avec des réservoirs du parasite dans la nature, même hors de l'existence de l'homme quelquefois. Dans ces conditions, nous avons fait nos recherches sur des rongeurs sauvages pour voir si le parasite existe aussi chez eux. Les recherches sont faites sur des rats et des souris sauvages et sur des *Citellus citellus*. De ces recherches faites sur 35 souris sauvages, 19 rats sauvages et 7 *Citellus citellus* nous avons constaté la présence du parasite chez un *Citellus citellus* provenant de la région de Sozopole. Dans ces conditions, des recherches sur les phlebotomes sont nécessaires, pour établir l'hôte vecteur du parasite en Bulgarie, ainsi que la propagation de la maladie parmi cette espèce animale *(Citellus citellus)*.

Ayant en vue le résultat de nos recherches sur la maladie, leishmaniose, ainsi que le grand pourcentage positif de recherches sérologiques sur la toxoplasmose (sans symptômes cliniques de la maladie), nous pensons que des recherches analogiques sont nécessaires pour établir la propagation de toxoplasmose dans les différents pays du monde et plus spécialement dans la Bulgarie et les pays voisins de la péninsule Balcanique.

# LA PROPAGATION DU KALA-AZAR EN YOUGOSLAVIE

MILAN GVOZDENOVIC [1]

RÉSUMÉ

Dans une communication précédente j'ai donné les dates principales qui concernent l'épidémiologie du kala-azar en Yougoslavie. En ces dernières années nous voyons que l'endémie du kala-azar gagne de nouveaux territoires et précisément vers le centre du pays, c'est-à-dire en Bosnie. C'est une région montagneuse et limitée par les montagnes assez hautes vers les régions litorales et vers les régions du sud, dans lesquelles l'endémie du kala-azar fut découverte depuis une trentaine d'années.

Les premiers cas du kala-azar en Bosnie ont été constatés après la deuxième guerre mondiale chez 2 adults et après parmi 6 enfants qui ont passé leurs vacances au bord de la mer, et précisément à Gradac. La faune de phlébotomines a été examinée quelques années auparavant au nord et au sud de ce lieu, et y sont trouvés en prévalence le *Ph. minutus* et le *Ph. major*. Il faut noter qu'aucune personne de la population indigène de ce lieu n'a pas souffert du kala-azar, à ce qu'en disent les médecins du lieu. Tandis que, une centaine de kilomètres au nord de cette région, et le long de la côte de la mer Adriatique, plusieurs cas de kala-azar ont été constatés dans le délais de temps entre les deux guerres mondiales. C'est donc seulement quelques nouveaux arrivés dans ce lieu qui ont été infectés avec les signes manifestes de la maladie, et précisément les infectés ont été les enfants provenants de la région montagneuse de la Bosnie. Deux de ces cas sont originaires de ce même lieu, mais ils venaient seule-

[1] The Author did not attend the meeting.

ment quelquefois pendant l'été pour y passer les deux mois des vacances, et les 4 cas ont passé seulement un mois de vacances dans ce lieu, et pendant ce delai de temps ils ont dû etre piqués par les vectors infectants. Cinq ans après nous voyons en Bosnie 2 cas de kala-azar infantile, un dans la région de Kakanj et l'autre dans la région de Fojnica. Ces deux lieux sont eloignés l'un de l'autre vers 70 km, mais séparés par un massive montagneux qui est très peu praticable et n'est pas peuplé, et, en surplus, ces deux lieus n'ont aucune communication directe. Ces deux enfants n'ont pas quitté son village avant d'avoir tombé malades, non plus leurs parents ne se sont pas eloigné plus loin de la petite ville, située dans la même région. Nous n'avons pas trouvé jusqu'à présent des phlébotomes dans ces villages, et non plus des chiens malades de kala-azar, quoique nos recherches dans cette direction ont été minutieuses, mais pendant un été pluvieux. Les travaux des recherches des nouvelles dates épidémiologiques continuent.

En face de nos investigations épidémiologiques, nous constatons que ces 2 cas de kala-azar sont autochtones et qu'ils ont quelque liaison avec les foyers endémiques sur le lieu cité du litoral. Nous voyons ainsi que l'endémie du kala-azar en Yougoslavie gagne de nouveaux territoires vers la région centrale, quoique montagneuse, du pays.

## GENERAL DISCUSSION

Dr. D. Berberian (U.S.A.) — I wish to discuss several epidemiological problems related to the transmission of oriental sore and infantile kala-azar in Lebanon and Syria.

Sporadic cases of cutaneous leishmaniasis began to appear in Beirut and in many villages in Lebanon where this disease was unknown prior to the First World War. The dissemination of the disease is no doubt ascribable to increased facilities for transportation and movements of people. Infections initially acquired in such hyperendemic foci as Bagdad and Aleppo were brought to Lebanon and there they developed and established new foci for further dissemination of the disease.

Sporadic cases of infantile kala-azar are also encountered in many parts of Lebanon. In the fall of 1946 two infants were brought to the hospitals of the American University of Beirut from Kessab, a small town in North Syria. They were diagnosed as kala-azar. Thereupon we decided to visit the area to examine the extent of the incidence. We examined all the children in the town and in the neighbouring villages, but failed to discover other cases. We also looked for cases of canine leishmaniasis. Ten stray dogs were shot and brought for examination. We were surprised to find two of the healthiest looking dogs infected. Numerous Leishman-Donovan bodies were found in their spleen and skin, yet they were both free of cutaneous lesions and their spleens were not markedly enlarged. Upon our return to Beirut we autopsied and examined more than 100 mongrel dogs and failed to find L-D bodies in their tissues.

The theory of the cyclical transmission of leishmaniases through the mediation of sandflies is a beautiful epic story. Nevertheless it does not fully meet the epidemiological observations in the field.

Even under the most optimum laboratory conditions it is possible to infect only a very small percentage of sandflies. Strains of leishma-

nias from different geographical locations differ markedly in their infectivity for different species of sandflies.

Lesions of cutaneous and muco-cutaneous leishmaniases are not *per se* attractive to sandflies. Admitting the possibility of sandflies accidentally landing on a lesion, their chances of becoming infected, of surviving the 10-12 day period necessary for the development and appearance of flagellates in their probosces, and of having the «aperitif» *(hors d'œuvres)* of raisin-juice before a successful bite are extremely small.

About 20 years ago I demonstrated that it was extremely easy to mechanically transmit oriental sore through the bites of stable flies. I am sure any other biting fly or arthropode would be able to transmit the disease through their soiled probosces. I urge my colleagues not to ignore this simple and unimaginative mode of transmission.

Dr. A. Corradetti (Italy) — I wish to ask Dr. Giraud two questions:

1. Which species of *Phlebotomus* are present in the zone of South France he studied? From our experience in Italy we have found always the presence of *Ph. perniciosus* everywhere cases of kala-azar occurred.

2. Had he any occasion to find babies under 1 year of age actually bitten by ticks? Does he hnow any case of kala-azar proved to have been actually transmitted by ticks?

Dr. A. Corradetti (Italy) — I wish to add some notes on the subject of leishmaniasis in Italy.

1. The geographical distribution of kala-azar in Italy is not limited to Southern Italy and Islands. About 100 cases were recently reported to have occurred in the last 20 years in Liguria (Genova and Imperia) only. Other foci I had occasion to find in Tuscany (Perugia, Siena, Monte Argentario).

2. The incidence of cutaneous leishmaniasis is much higher than described in the paper. In 1948 I examined the entire population of a district 25 km long and 15 km wide in Abruzzo. There were examined about 29,000 people. More than 2 % showed active lesions and 21 % had the scars.

3. The geographical distribution of *Phlebotomus* species and leishmaniasis in Italy shows a close relation of *Ph. perfiliewi* with oriental sore and of *Ph. perniciosus* with kala-azar.

Dr. Georges Blanc (Maroc) — À propos de la communication du Prof. Giraud le Dr. Georges Blanc rappelle ses expériences faites il y a 25 ans à Athènes sur la transmission du kala-azar par les tiques *(Rhipicephalus sanguineus)*. Ayant constaté l'extrême sensibilité du spermophile au kala-azar, il fait piquer ces rongeurs infectés expérimentalement par des larves de *Rh. sanguineus,* celles-ci s'infectent et après la mue, soit à l'état de nymphes, elles sont encore infectées. De plus, il constate que des rhipicephales adultes trouvées gorgées sur des chiens atteints de kala-azar sont, elles aussi, infectées, d'où l'opinion qu'il exprime que les rhipicephales des chiens peuvent jouer, surtout chez l'enfant, un rôle important dans la transmission du kala-azar, au moins par écrasement et contamination par la peau ou les muqueuses.

Dr. L. A. Stauber (U.S.A.) — In the reported experiment where Prof. Adler injected leishmaniae into the immune and attempted recovery of the organisms in culture at intervals after the superinfection: *a*) how many organisms were used in the superinfection? *b*) were other samples removed and what were the probable percentages of organisms killed at these intervals?

Prof. S. Adler (Israel) — The large amounts of flagellates *L. tropica* inoculated into an immune subject disappeared within 24 hours, although a few survived and were demonstrated by culture. After 48 hours it was no longer possible to demonstrate them.

Dr. Giroud has pointed out the typical age distribution of cases of *L. infantum* and he associates it with crawling on the ground and contact with ticks. A more feasible explanation is the presence in adults of a globulin which destroys *L. infantum* and its absence in children up to the age of 6 or 7.

There is no correlation between the distribution of *L. infantum* and *Rhipicephalus sanguineus* or any other tick, but there is a close correspondence between the distribution of the disease and various species of *Phlebotomus*.

Dr. P. Pavlov (Bulgaria) — *Réponse à la question posée:* Si j'ai bien compris la question de mon collègue, je peux lui donner la réponse suivante:

1. La maladie chez l'homme est constatée en cas isolés. C'est seulement dans la région de Vraza qu'on a constaté 40 cas de la maladie chez les enfants.

2. Chez le chien la maladie existe surtout dans la Bulgarie du Sud, près de la frontière grecque.

De 120 chiens examinés 48 ont réagi sérologiquement. De ces 48 chiens 10 % ont montré la présence du parasite — c'est-à-dire, 10 % de 48=4,8.

De tous les 120 examinées, 4,8 % de 48, ça fait un pourcentage de 0,4 de 120 chiens examinés. C'est un résultat reçu en Bulgarie du Sud.

Probablement dans les autres régions du pays le pourcentage sera encore plus bas.

Prof. Jacques Ranque (France) — À propos du diagnostic du kala-azar canin, il est un bon moyen de prospection qui est la ponction ganglionnaire facilement positive au début de l'infestation.

À la période d'état les lésions cutanées sont le plus souvent évidentes, mais il est très difficile de trouver les leishmanies dans la peau par suite d'une forte hyperkératose. D'autre part, il est difficile de faire accepter aux propriétaires de chiens la ponction de rate, organe allongé se prêtant mal à cette manœuvre.

Du point de vue sérologique, on note toujours à la période d'état un thymol test de Mac Lagan fortement positif. C'est un signe de présomption qui incitera à chercher plus longtemps le parasite dans les ganglions ou dans la moelle osseuse.

2ND SESSION: **American leishmaniasis**

*Tuesday, September 9th — 9:00 to 10:30 a. m. Room C*

The Chairman of the meeting was Dr. Saul Adler and the Rapporteur was Dr. Samuel Pessoa. The Secretary was Dr. Maria do Pilar F. Gomes.

The following papers were presented and discussed.

## LEISHMANIOSE TEGUMENTAR AMERICANA

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### DISTRIBUIÇÃO GEOGRÁFICA

A presença da leishmaniose tegumentar tem sido assinalada em quase todo o hemisfério ocidental, desde o México até o norte da República Argentina. Pouco frequente em muitos países, a moléstia apresenta alta incidência em certas regiões de alguns países da América do Sul, em particular do Peru e do Brasil. Casos autóctones foram assinalados nos seguintes países: Martinica, México, onde é denominada «úlcera dos *chicleros*», Honduras Britânicas, Honduras, Guatemala, Costa Rica, Salvador, Nicarágua, Panamá, Venezuela, Colômbia, Guianas, Equador, Peru, Bolívia, Paraguai, Argentina e Brasil. Não é autóctone no Canadá, Estados Unidos, Uruguai e Chile.

No Brasil a leishmaniose está espalhada em todo o território nacional. Certas regiões, como o estado de São Paulo, foram grandes focos de leishmaniose tegumentar; porém, com a derrubada das matas

e formações de fazendas (plantações), a doença vem perdendo a sua importância como problema de saúde pública.

## ETIOLOGIA

Os primeiros autores que estudaram a moléstia existente na América identificaram-na ao botão-do-oriente. Descoberto o seu agente etiológico por Lindenberg (1909) e Carini e Paranhos (1909), foi considerado idêntico à *Leishmania tropica* Wright (1903). Posteriormente, Gaspar Viana (1911), estudando o parasita, propôs fosse considerado espécie distinta, denominando-o *Leishmania brasiliensis,* o que é aceito até hoje. Biagi (1953) prefere considerar o agente etiológico da leishmaniose mexicana como determinado por uma subespécie da *Leishmania tropica,* que ele denominou de *Leishmania tropica mexicana.*

A *Leishmania brasiliensis,* como as demais espécies do género, preenche seu ciclo na natureza em dois hospedeiros: o vector, díptero, sugador de sangue, do género *Phlebotomus;* e outro vertebrado — que pode ser o homem. Esse é o ciclo conhecido até agora, pouco se sabendo no Brasil, e em geral no continente americano, sobre os reservatórios do parasita. A *L. brasiliensis* apresenta-se morfològicamente sob duas formas fundamentais: uma aflagelada — a forma em leishmânia, encontrada no hospedeiro vertebrado — e uma flagelada — a forma em leptomonas, observada no tubo digestivo do insecto vector e também nas culturas do parasita. Estas formas transformam-se, reversìvelmente, uma na outra e entre elas existem todos os graus de transição.

## EPIDEMIOLOGIA

### Generalidades

A leishmaniose tegumentar americana predomina nas zonas húmidas florestais. Não é observada em regiões áridas e secas, a menos que em determinadas épocas do ano se registrem microclimas favoráveis ao ciclo biológico dos insectos vectores. Nas regiões meridionais a altura limita sua endemicidade. Pessoa e Pestana (1941) nunca

assinalaram-se casos em altitudes maiores que 1000 m, no Sul do Brasil. À medida que se aproxima do Equador, a altitude perde sua importância, pois existem zonas endémicas de uta (leishmaniose cutânea) no Peru, em áreas situadas entre 1000 m e 2800 m sobre o nível do mar. Na Venezuela, Pifano (1950) encontrou doentes em lugares a 1500 m e 1800 m de altitude.

Ainda que se possa adquirir a moléstia em todas as épocas do ano, as melhores condições de transmissão parecem realizar-se depois da estação chuvosa.

Ataca a protozoose indistintamente qualquer idade, sexo ou raça; as variações estatísticas que se podem observar em tal sentido são aparentes e se devem à maior ou menor exposição às picadas dos insectos vectores, de acordo com a profissão (trabalhos florestais) e a localização das habitações mais ou menos próximas das matas.

**Transmissão**

Em geral, os autores admitem ser a leishmaniose tegumentar transmitida nas condições naturais pela picada de pequenos dípteros psicodídeos, sugadores de sangue, denominados *Phlebotomus*. Foi Aragão (1922) quem, pela primeira vez no continente americano, demonstrou o papel vector do *Phlebotomus intermedius*. Posteriormente, Pessoa, em colaboração com Pestana, Barreto e Coutinho, demonstraram a importância epidemiológica em zonas endémicas do estado de São Paulo das seguintes espécies de *Phlebotomus:* o *P. migonei,* encontrado infectado na natureza na proporção de 0,21 %; o *P. withmani,* 0,20 %; e o *P. pessoai,* 0,29 %. Além destes, Forattini e Santos (1950) encontraram o *P. intermedius* infectado na natureza — 0,14 %. Na Venezuela, Pifano (1954) encontrou as seguintes espécies naturalmente infectadas: *P. panamensis, P. migonei* e *P. longipalpis*. Para Floch (1957), na Guiana, o *P. anduzei* parece ser o vector natural. A prova crucial, porém, da transmissão da leishmânia pela picada do flebótomo ainda não foi obtida. Assim, pode-se também admitir a possibilidade da transmissão por outros vectores, todavia desconhecidos, ou a da transmissão mecânica, por picada interrompida ou não, no caso em que os germes sejam suficientemente resistentes para viver na tromba do insecto hematófago, até que uma nova picada seja feita no homem ou no animal sensível.

No estado actual dos nossos conhecimentos podemos aceitar a hipótese de se dar a principal transmissão por meio de flebótomos. Esta hipótese explica, em geral, a epidemiologia da doença na América. Assim, as espécies infectadas até hoje encontradas no Brasil apresentam hábitos exclusivamente silváticos ou semidomésticos. Vivem, de preferência, nas florestas virgens, em que o grau de humidade e o calor facilitam o seu desenvolvimento. O suporte biológico da doença é constituído pelas florestas, xerófilas ou não, ou matas húmidas e quentes, com árvores derrubadas, troncos, etc. Pelo facto de não se desenvolverem as espécies transmissoras perto das habitações, vemos que a moléstia não é encontrada nas cidades e vilas, mesmo as situadas dentro de regiões altamente endémicas. No entanto, condições microclimáticas favoráveis determinam o aparecimento de casos na vizinhança de cidades, como foi descrito por Pessoa e col. em Araçatuba e Pessoa e Pestana nos arredores de São Paulo. As zonas mais atingidas são, porém, as regiões florestais. Também a densidade dos vectores é importante. Como a taxa de infecção natural dos flebótomos é pequena, cerca de 0,2 %, como vimos, é necessário, para haver a transmissão, sejam os indivíduos picados por número considerável destes insectos. É realmente o que se verifica nas zonas endémicas: os flebótomos formam verdadeiras nuvens, que invadem as habitações situadas na região florestal em certas épocas do ano, sendo seus habitantes picados por milhares de tais insectos durante vários meses.

**Animais sensíveis**

Não são muito extensos nossos conhecimentos sobre a questão dos reservatórios vertebrados extra-humanos da *Leishmania brasiliensis* nas zonas endémicas. Há razões experimentais e epidemiológicas que tornam imprescindível a existência de um depositário animal no ciclo da leishmânia. Só assim poderíamos explicar a aparição de numerosos surtos verdadeiramente explosivos na exploração de florestas virgens de população humana até àquele momento. A infecção espontânea do cão foi comprovada primeiramente no Brasil por Pedroso (1912) e, posteriormente, em outros lugares e por vários autores.

Herrer (1948), no Peru, em localidades onde a leishmaniose tegumentar é endémica, encontrou 40 cães infectados entre 469 examinados. Não verificou neles lesão ulcerosa visível; eram portadores

de perturbações da pele sob a forma de alopecias e acromias, localizadas, habitualmente, no focinho e nas orelhas.

No Brasil o papel epidemiológico do cão, de acordo com Pessoa e Barreto (1946) e Forattini e col. (1957), parece ser pequeno, se não desprezível, pois é encontrado raramente infectado e, em geral, com número escasso de parasitas nas lesões; não se comporta, pois, como verdadeiro reservatório.

Recentemente Hertig, Fairchild e Johnson (1957) isolaram o parasita de ratos silvestres no Panamá. Fazendo cultura do sangue de uma espécie de rato selvagem, muito comum na região, o *Proechymys semispinosus panamensis,* conseguiram cultura da leishmânia a qual, inoculada em voluntário humano, determinou uma lesão cutânea típica na qual foram demonstrados os parasitas. E interessante, em conexão com este estudo, foi o facto de, apesar de a *Leishmania* ter sido isolada do sangue de vários ratos pela cultura, em nenhum deles foi possível a demonstração do parasita nos tecidos pelos processos habituais do exame histológico microscópico.

No estado de São Paulo, Forattini e col. (1958) obtiveram hemoculturas positivas para *Leishmania* a partir de sangue circulante de roedores silvestres (rato: *Kannabateomys amblyonyx,* e paca: *Cuniculus paca paca*). No primeiro observaram uma pequena ulceração no dorso do focinho, a qual se revelou positiva para leishmânias.

## PATOLOGIA

### Generalidades

Na generalidade dos casos, a infecção do homem pela *L. brasiliensis* se estabelece em consequência da inoculação de formas em leptomonas pela picada dos flebótomos em partes descobertas da pele. Após uma fase de incubação, durante a qual os parasitas se multiplicam, sob a forma de leishmânia, nos elementos histiocitários da pele, aparece a lesão inicial, revestindo, em geral, a forma de uma pápula ou eritema papuloso, em cujos elementos pululam os corpúsculos parasitários. Tais lesões primitivas podem regredir, apresentando um decurso abortivo que até o momento tem sido mal conhecido. Para Floch e Casile (1955) tais formas abortivas, que denominam *subclini-*

*cas,* são, em certas circunstâncias, bastante frequentes. Verificaram tal facto submetendo 64 pacientes vivendo na floresta, porém não mostrando cicatrizes, à intradermorreacção de Montenegro. Apresentaram reacções positivas 47 %, enquanto, entre 37 outros indivíduos da região, que, porém, não frequentavam nem viviam na floresta, nenhum mostrou reacção positiva. Para os autores citados, desde que a reacção de Montenegro é prova específica para a leishmaniose tegumentar, este resultado mostra que, no primeiro grupo, os indivíduos se tornaram alérgicos devido a uma infecção prévia e subclínica, que, como se vê, é bastante frequente.

De outro lado, as lesões, progredindo, darão formação a lesões ulcerosas ou vegetantes. Em seguida, por mecanismo não claramente elucidado, os parasitas, por metástases da lesão primária, invadem as mucosas da região nasobucofaringolaríngea, determinando alterações ulcerosas ou vegetantes de carácter crónico e raramente com tendência à cura espontânea.

Como certos autores (Guimarães, 1947, 1951; Mohiuddin, 1952; Muniz, 1953) conseguiram por meio de injecções em animais de laboratório a visceralização da *L. brasiliensis,* pretendeu-se concluir sobre a identidade dos parasitas da doença cutânea e visceral, não passando, pois, a *L. tropica* e a *L. brasiliensis* de raças biológicas da *L. donovani* (Mohiuddin, 1952). Ora só no estado de São Paulo, em que a leishmaniose tegumentar reinou endemo-epidèmicamente durante mais de 50 anos, ocasionando centenas de milhares de casos, nunca se registrou um único doente de calazar autóctone do estado em apreço. Assim, se experimentalmente e em animais de laboratório os parasitas se comportam idênticamente quanto ao dermo e viscerotropismo, na prática as duas doenças, a leishmaniose tegumentar e a visceral, se apresentam, constante e nìtidamente, como duas afecções perfeitamente distintas.

### Incubação

O período de incubação parece ser, em média, de 2 semanas a 1 a 2 meses; pode, todavia, haver casos isolados nos quais esse período se encurte para dias ou se alongue para um ano ou mais.

### Lesões iniciais

As lesões iniciais são individualizadas pela pápula vesiculosa, a despeito de essa lesão não ser obrigatória, sendo frequentes os casos de lesões frustas ou disseminadas em que não é observada a fase primária da moléstia. No início verifica-se, muitas vezes, uma adenopatia satélite, sem processo intermediário de linfangite; esta adenite tem tendência resolutiva, desaparecendo no fim de 1 a 2 meses. Assim, a lesão leishmaniótica inicial constitui um verdadeiro complexo cutâneo-ganglionar. O exame parasitológico da lesão inicial demonstra grande quantidade de corpúsculos parasitários, também encontrados precocemente nos gânglios ingurgitados. Processa-se a ulceração desta pápula inicial, em virtude de necrose com sede no derma, atingindo precocemente o epitélio, que, lesado, acaba por descolar-se; toma a lesão feição ulcerosa típica (cancro leishmaniótico) e a ela sucedem-se outras lesões, dentro do período de 1 a 2 meses em que elas se multiplicam sob carácter regional ou distal, podendo ainda haver disseminação hematogénica dos parasitas e a moléstia se apresentar sob o tipo eruptivo e com múltiplos quadros clínicos.

### Formas clínicas

E. Rabelo (1925), a quem se deve uma classificação, hoje clássica, das formas clínicas da leishmaniose tegumentar, distinguia desde logo as lesões por uma característica fundamental, que é a presença ou ausência de solução de continuidade da pele — formas ulcerosas e não ulcerosas. Num mesmo paciente, porém, podem-se encontrar lesões de ambos os aspectos.

Formas ulcerosas.

São as mais frequentemente observadas. Podem ser rasas, lembrando o éctima, e daí a terminologia *lesão ectimatóide*, ou são mais profundas, usando-se então a designação *ulcerosa franca*. Nestas lesões, as bordas ora são regulares, salientes, intensa e largamente infiltradas, ora são irregulares, pouco salientes e recobertas de crostas córneas. O exsudato, seroso no início, apresenta-se purulento

quando a infecção bacteriana é intensa; sob as membranas pardacentas que recobrem o fundo, e que se situam ao nível da pele circunjacente, fica estagnado o secreto purulento. Destacada a membrana e removidas as secreções, o fundo se mostra vermelho-vivo e com granulações finas, sangrando fàcilmente. Casos há em que o fundo da úlcera é grosseiramente granuloso, pela formação de botões carnosos — são as lesões *ulcerovegetantes.*

Lesões não ulcerosas.

*a*) *Impetiginóides.* — Em certos casos encontram-se lesões que lembram o impetigo; trata-se de uma manifestação papulopustulosa. Podem elas confluir e, ao romper-se, dão lugar à formação de crostas, que, levantadas, mostram a presença de exulceração.

*b*) *Liquenóide ou forma papulofolicular.* — As lesões podem desenvolver-se ao redor dos folículos pilosos ou sebáceos, exteriorizando-se por pequenas pápulas de 2 mm a 3 mm, às vezes confluentes em placa ou em coroa; às vezes são generalizadas e dependem de um mecanismo hematógeno, motivo por que Aguiar Pupo as classifica sob a denominação de *leishmânides.*

*c*) *Tuberosa.* — Podem-se distinguir as lesões macrotuberosas e as microtuberosas; em ambas o aspecto é francamente lupóide, como as classifica F. E. Rabelo. Quanto às microtuberosas, descritas por Prates, Portugal e Rutowitch (1943), parecem ser de origem hematogénica, localizando-se preferentemente na face.

*d*) *Nodular ou dermipodérmica.* — No Brasil tal forma parece excepcional; não conhecemos documentação fotográfica desta lesão. É mais comummente encontrada na América Central.

*e*) *Formas vegetantes.* — Podem ser de dois tipos: framboesóides e verrucosas. As primeiras são formações com aspecto de couve-flor, de extensão variável e podendo atingir, acima da pele vizinha, uma altura de 2 mm a 3 mm. A superfície da lesão sangra fàcilmente, apresentando-se, não raro, recoberta por exsudatos espessos ou crostas. As verrucosas, em geral mais ou menos nìtidamente delimitadas das regiões vizinhas da pele, apresentam-se como elevações de pequena altura (cerca de 1 mm), ásperas, rugosas, semelhantes às verrugas. São formas de resistência da moléstia.

Muitas vezes simulam a dermatite verrucosa (cromoblastomicose), as lesões antigas da bouba ou a blastomicose brasileira (granuloma paracoccidióidico). São as formas cromoblastomicóides e framboesiformes de Pifano e col. (1954).

Formas linfáticas.

Tanto a linfangite como a adenite ocorrem frequentemente na leishmaniose tegumentar. O vaso linfático engrossa, transforma-se em cordão espesso de diâmetro uniforme, não doloroso; em geral, não há hiperemia e apresenta, muitas vezes, nódulos bem nítidos no seu trajecto, palpáveis através da pele. Em certos casos, estes nódulos podem romper-se, dando origem a novas úlceras em disposição linear. Tais formas podem ser confundidas com a esporotricose: daí serem denominadas de *formas esporotricóides* por Pifano e col. (1954).

**Multiplicidade e localização das lesões**

Já Lindenberg (1909) assinalara que a leishmaniose geralmente se inicia com uma única lesão, aparecendo outras semanas ou mesmo meses depois. Em certos casos, porém, várias lesões se manifestam simultâneamente. Pacientes com numerosíssimas lesões, embora raros, têm sido observados. É possível que nestes casos os parasitas se disseminem por via hematogénica; entretanto, apesar de numerosas tentativas, nunca se conseguiu evidenciar a presença de leishmânias no sangue.

Quanto à localização das lesões, todos os autores estão de acordo em assinalar, como sede preferencial, as partes descobertas do corpo, em particular os membros inferiores e superiores e a cabeça.

Interessante é notar que, enquanto no Brasil e na Venezuela a leishmaniose tem predilecção pelos membros inferiores, no México (Beltran e Bustamante, 1942) e em Costa Rica (Peña Chavarria, 1937) tem ela predilecção para se localizar nas orelhas, em seguida no braço, sendo que o número de feridas que se encontram nos pés ou nas pernas é muito pequeno.

É possível que diferenças na biologia do flebótomo vector expliquem as divergências apontadas. De qualquer forma, é fora de dúvida

que a localização primária decorre da maior ou menor exposição desta ou daquela parte do corpo à picada dos vectores.

### Lesões mucosas

Um dos caracteres mais típicos da leishmaniose tegumentar americana, e precisamente aquele em virtude do qual a moléstia assume gravidade, é a frequência com que os parasitas invadem a mucosa nasofaringolaríngea, determinando lesões destrutivas, não raro de proporções assombrosas. Neste particular difere do botão-do-oriente, em que a localização mucosa é excepcional. O mecanismo da invasão das mucosas pela *L. brasiliensis* não está perfeitamente esclarecido. Alguns autores admitem a possibilidade de uma auto-inoculação exógena; a via hematogénica, defendida por outros, é a mais aceitável, embora não se tenha ainda conseguido observar leishmânias na circulação. Vilela, Pestana e Pessoa (1939) constataram parasitas em mucosa do nariz aparentemente integra; posteriormente Pifano (1945), na Venezuela, confirmou tais achados.

O processo nasal se inicia com uma hiperemia circunscrita da mucosa, geralmente localizada na parte anterior do processo cartilaginoso, seguindo-se uma pequena infiltração que aparece como pontos esbranquiçados do tamanho de uma cabeça de alfinete, às vezes isolados, outras vezes múltiplos. Nesta fase o doente nada sente; em certos casos acusa sintomas de coriza. Com o correr do tempo, às vezes precocemente, o processo evolui para a ulceração. A úlcera aumenta em profundidade e extensão, atinge a cartilagem septal e a mucosa do lado oposto do septo se infiltra e ulcera. Em consequência, temos a perfuração do septo e, posteriormente, a destruição do subsepto. As lesões não se limitam ao septo membranoso; acabam, mais cedo ou mais tarde, por atingir toda a armadura cartilaginosa do nariz. As partes externas são comprometidas. A princípio a pele se torna avermelhada por uma telangiectasia superficial, logo depois infiltrada, espessa, rija e rugosa como em um rinofima, resultando daí o aspecto conhecido por «nariz de tapir» ou «focinho de anta». Nos casos mais avançados o dorso do nariz é perfurado e a ulceração se estende, deformando horrìvelmente a fisionomia do indivíduo. Todavia, as partes ósseas não são comprometidas. O processo pode atingir, por continuidade, o lábio superior, assim como o inferior, e

se estender pela face, resultando mutilações pavorosas. As lesões nasais podem-se estender e invadir a rinofaringe, o palato, as paredes póstero-laterais da faringe e, eventualmente, a laringe, a língua e as gengivas. Como localizações excepcionais podemos citar: a árvore traqueobrônquica, a língua, a mucosa genital, a mucosa ocular, etc.

Uma forma mucosa bem estudada por Mangabeira Albernaz (1945 e 1947) é a poliposa. Os pólipos leishmanióticos, pràticamente indistinguíveis dos pólipos comuns, localizam-se, em geral, no septo nasal.

**Frequência das lesões mucosas**

Varia muito a opinião dos diversos autores quanto à frequência com que a leishmaniose tegumentar atinge as mucosas. Alguns autores, como Brumpt e Pedroso (1913), registraram lesões mucosas em cerca de 20 % dos enfermos; Klotz e Lindenberg (1923) e Barbosa (1936) em 15 a 20 % dos enfermos; em uma primeira estatística de Pestana, Pessoa e Correia, em 13 %. Posteriormente Pessoa (1941) encontrou lesões mucosas em 38,5 % dos doentes com lesões cutâneas com menos de 1 ano e em 80,9 % dos que apresentavam lesões cutâneas com mais de 1 ano.

A localização nas mucosas parece variar não só com o tempo da doença, mas também com as regiões geográficas, pois no México (península de Yucatan) não se registam lesões das mucosas na leishmaniose.

**Diversidade clínica da leishmaniose tegumentar americana nas várias regiões geográficas**

Biagi (1953*a* e 1953*b*) distingue as seguintes variedades de leishmanioses tegumentares: 1) Leishmaniose tegumentar mexicana, encontrada no México e na Guatemala, caracterizada por lesões ectimatóides, localizadas principalmente nas orelhas. Lesões metastáticas pouco frequentes; lesões do sistema linfático escassas; lesões mucosas inexistentes, a não ser por contiguidade de uma lesão cutânea. 2) A leishmaniose tegumentar do Norte da América do Sul (uta), encontrada desde o Peru até as Guianas e Costa Rica, caracterizada por apresentar formas ulcerosas pequenas que se curam espontâneamente. Maior número de lesões linfáticas (10 %), não determinando

lesões mucosas. a não ser por contiguidade. 3) A leishmaniose mucocutânea (espundia), encontrada no Brasil e países vizinhos, como Paraguai, Argentina, Bolívia, etc. Apresenta, frequentemente, lesões cutâneas ulcerosas francas, tendência à cronicidade e elevada percentagem de invasão das mucosas, não só por contiguidade, mas principalmente por metástase. A linfadenite mostra-se pouco frequente.

Para Floch (1954, 1957) estas três variedades de leishmaniose seriam determinadas por três agentes etiológicos diferentes, respectivamente a *Leishmania tropica mexicana* de Biagi (1953), a *Leishmania tropica guyanensis* e a *Leishmania tropica brasiliensis*. Como faz notar Hoare, se tal nomenclatura for aceita, o agente do botão-do-oriente se denominará *Leishmania tropica tropica*.

Para Jaffé (1954), entretanto, a mesma doença ocorreria do México à Argentina. As lesões das mucosas, especialmente as nasais, são mais frequentes nos países meridionais do Novo Mundo e menos frequentes nos setentrionais. Este autor, só no Panamá, diagnosticou 8 casos de lesões mucosas nasais; mostrou Jaffé a dificuldade do diagnóstico destas lesões, sendo que, em muitos casos, são erròneamente tomados como tuberculose. Os diferentes aspectos da doença seriam pois de natureza quantitativa, e não qualitativa, como se dá para qualquer moléstia infecciosa nas várias regiões geográficas; o agente etiológico seria, entretanto, o mesmo.

**Formas atípicas da leishmaniose tegumentar americana**

Vários autores, como Convit e Lapenta (1948) na Venezuela, Barrientos (1948) na Bolívia, Convit e col. (1957) e outros citados por Convit e col. (1957). descreveram formas atípicas, difusas e graves da leishmaniose mucocutânea. Tais casos se caracterizam, de maneira geral, pela tendência à difusão das lesões cutâneas, pela falta de reactividade ao antígeno de Montenegro e pela abundância extraordinária de leishmânias nas lesões. Apesar da resistência dos parasitas a todos os agentes terapêuticos conhecidos até hoje, o estado geral do paciente não é muito afectado. O quadro anátomo-patológico é a atrofia da epiderme com hiperceratose moderada. O derma apresenta granulomas formados por células com grandes vacúolos abarrotados de leishmânias.

## ANATOMIA PATOLÓGICA

### Lesões iniciais

Caracterizam-se por uma infiltração linfoplasmistiocitária do derma e proliferação do epitélio que o recobre e, nas lesões mais antigas, uma extensa formação de crostas. O epitélio mostra uma acentuadíssima acantose, sob a forma de cordões epiteliais que invadem o derma infiltrado. No derma observa-se uma infiltração linfoplasmistiocitária, variável de acordo com a lesão. Assim, às vezes os plasmócitos dominam o quadro, facto que levou Montenegro (1924) a descrevê-los como verdadeiros plasmomas. Em outros casos os linfócitos são tão abundantes quanto os plasmócitos. Também se encontram infiltrados focais constituídos por um acúmulo central de células histiocitárias rodeado de plasmócitos e linfócitos — são as chamadas *clareiras* de Montenegro ou *centros claros* de Buss (1929). Nesta fase não se observam estruturas tuberculóides nítidas nem gigantócitos.

### Formas ulcerosas

Nas lesões pequenas o epitélio das margens apresenta alterações de pouca monta, consistindo em espessamento e acantose. Nas úlceras grandes há uma acantose acentuada, que, sob a forma de cordões epiteliais, invade o derma infiltrado; a camada espinhosa mostra-se espessada; não há camada granulosa e a camada córnea exibe paraceratose; encontram-se pérolas córneas e, às vezes, papilomatose de grau moderado. O derma mostra-se infiltrado e os capilares congestos e verifica-se infiltração celular rica em polimorfonucleares neutrófilos. Células gigantes e estruturas tuberculóides são encontradas nas úlceras crónicas, onde aparecem nódulos tuberculóides bem nítidos. Os fibroblastos faltam nas úlceras recentes, sendo notados cada vez em maior número nas úlceras mais antigas com tendência à cicatrização.

### Formas não ulcerosas

Nas lesões tuberiformes nota-se uma infiltração linfoplasmistiocitária do derma e proliferação do epitélio que o recobre. O epi-

télio mostra acantose e pérolas córneas. Crostas formadas por lâminas paraceratósicas e exsudato seroso podem recobrir o epitélio mais ou menos lesado.

#### Lesões verrucosas e papilomatosas

Não há, do ponto de vista histológico, diferenças essenciais destas duas formas. A camada epitelial se mostra sempre grandemente espessada. O extracto espinhoso também se apresenta bastante espessado e há formação de grandes massas córneas que se acumulam nas depressões interpapilares. A proliferação pode invadir desordenadamente os tecidos subjacentes.

#### Lesões mucosas

Foram bem estudadas por Klotz e Lindenberg (1933), que assinalam a formação de nódulos circunscritos de células histiocitárias como o elemento mais característico da fase de ulceração.

## DIAGNÓSTICO

#### Diagnóstico clínico

O diagnóstico clínico da leishmaniose cutânea não apresenta, em geral, maiores dificuldades quando assumem as lesões o aspecto ulceroso típico, bastando, muitas vezes, um exame objectivo para estabelecer a sua natureza. As formas vegetantes e linfangíticas podem ser confundidas com outras entidades nosológicas, tornando-se difícil firmar o diagnóstico, a não ser pelos métodos laboratoriais.

Quanto à forma mucosa da moléstia, a anamnese é bem importante, pois a maioria dos doentes com lesão mucosa já se referem a lesões da pele. Quando estas não são presentes, podemos encontrar suas cicatrizes. A localização da lesão que se assenta sempre na porção cartilaginosa do septo, bem como seu aspecto geral, ajudam-nos no diagnóstico. O edema local e a hipertrofia, principalmente no lábio superior, emprestam ao doente uma fisionomia especial que alguns autores denominam *fácies leishmaniótica* e outros *focinho de anta*.

### Diagnóstico de laboratório

Demonstração do parasita.

A demonstração do agente causal pode ser feita quer pelos métodos directos, isto é, sua pesquisa na úlcera, gânglio, etc., quer pela cultura dos mesmos. Nas lesões atrás citadas o material obtido por curetagem, punção ou biopsia é semeado em meio de N. N. N. ou com ele são feitos esfregaços em lâmina. Nas lesões recentes é fácil a demonstração do parasita por simples esfregaços e coloração posterior pelo método de Giemsa. Já nas ulcerações mais antigas, contaminadas por diversos germes, bem como nas lesões das mucosas, os parasitas são encontrados em número escasso, sendo laboriosa a comprovação dos parasitas. Como a leishmânia não se desenvolve em presença de qualquer outro germe, usa-se semear o material em muitos tubos ou juntar penicilina ao meio.

Métodos indirectos.

As reacções sorológicas usadas para o diagnóstico do calazar não dão resultados satisfatórios na leishmaniose mucocutânea.

O mais usado dos métodos indirectos é a reacção intradérmica de Montenegro. A intradermorreacção é uma reacção alérgica, introduzida na prática médica por Montenegro (1926). O antígeno, constituído por uma suspensão de 4 a 5 milhões de leptomonas por centímetro cúbico em solução salina fenolizada, é aplicado em injecções intradérmicas na dose de 0,10 $cm^3$ a 0,15 $cm^3$. Nos casos positivos verifica-se a formação de uma pápula, que atinge o auge no curso de 28-72 horas, e assim permanece durante 4-5 dias, para regredir e desaparecer lentamente. Tal prova nos fornece alta percentagem de positividade (95 a 97 %); pode ser negativa nas lesões recentes; porém estes casos fornecem sempre resultado positivo quanto à pesquisa de parasitas. Rotberg (1952) julga útil a leitura de rotina da reacção de Montenegro em duas épocas: a primeira após 48-72 horas, como de hábito, e a segunda aos 15 dias. Não crê este autor que a fase tardia seja apenas uma simples «permanência» ou «resíduo» da reacção precoce, mas sim fenómeno à parte.

A positividade da reacção perdura por muitos anos, quiçá por toda a vida, apesar da cicatrização e cura da leishmaniose.

## TRATAMENTO

### Tratamento local

Como terapêutica local usam-se substâncias desinfectantes, ácido láctico ou injecções de atebrina na base das úlceras. Também têm sido empregados, principalmente nas lesões mucosas, o termocautério, a diatermofulguração e a diatermocoagulação.

Tais processos locais, meramente adjuvantes, devem ser sempre associados ao tratamento específico geral.

### Tratamento geral

Sais de antimónio.

*a*) *Tártaro emético.* — Coube a Gaspar Viana a glória de descobrir um medicamento eficiente para a cura da leishmaniose. Em 1912 Gaspar Viana comunicou que o tártaro emético a 1 % determinava rápida cicatrização das lesões cutâneas, curando alguns enfermos e melhorando vários outros.

Na leishmaniose tegumentar o tártaro emético deve ser usado em injecções intravenosas em solução aquosa a 1 ou 2 %.

*b*) *Fuadina (Neo-antimosan).* — Inicialmente fabricado pela casa Bayer, depois da guerra começou também a ser fabricado nos Estados Unidos pela casa Winthrop, sob o nome de *Repodral*. É um sal sódico de antimónio, contendo 13,5 % de antimónio. É administrado por via intramuscular em dias alternados. A grande vantagem da Fuadina sobre o tártaro emético é a sua aplicação por via intramuscular.

*c*) *Anthiomaline.* — É o antimónio-tiomalato de lítio, preparado pela Rhodia. O seu uso é intramuscular, sendo feitas aplicações em dias alternados nas doses de 2 $cm^3$ a 3 $cm^3$.

*d*) *Glucantime.* — A Glucantime, antimonial pentavalente, à base de N-metil-glucamine, tem sido utilizada com bons resultados (Correia e Morgante, 1956). Pode ser empregada por meio de injecções intra-

musculares ou intravenosas, diárias ou em dias alternados, na dose de 5 $cm^3$ a 10 $cm^3$. Outros empregam injecções de 20 $cm^3$ durante 10 dias (4 ampolas de 5 $cm^3$). Poderão ser feitas duas ou mais séries com intervalos de 1 semana.

Sais de arsénico.

Aguiar Pupo (1926) introduziu, com sucesso, o Eparseno (amino--arseno-fenol) na terapêutica da leishmaniose tegumentar. Cada ampola de 1 $cm^3$ de Eparseno contém 0,12 g do sal; é usado em injecções de 1 $cm^3$, aplicadas com intervalos de 2 dias.

Aguiar Pupo também preconiza o uso do arsenito de sódio, na dose de 2 mg a 5 mg por injecções intramusculares.

Numerosos outros medicamentos preconizados (Sulfarsenol, Atebrina, Aralen, antibióticos, Sulfadiazina, etc.) não têm dado bons resultados. Pode-se dizer que o problema terapêutico da leishmaniose tegumentar deve ser ainda objecto de mais investigações.

Diamidinas aromáticas.

A mais usada pelos clínicos é a Lomidina (diamidina-difenoxi--pentana). O medicamento é apresentado em solução de 2,5 %, para uso intramuscular. Como dose óptima são usadas 10 ampolas em injecções intramusculares em dias alternados.

## PROFILAXIA

O DDT veio, pràticamente, resolver o problema da profilaxia da leishmaniose. A dificuldade consiste na sua aplicação em certos tipos de habitações rurais das zonas endémicas em que residem os trabalhadores na mata, especialmente os mais expostos. Onde pôde ser ele aplicado, como, por exemplo, por Herrer no Peru (1956) e por Guimarães (1955) na Baixada Fluminense, em que se verificava transmissão domiciliária provàvelmente pelo *Phlebotomus intermedius*, deu-se a queda ou a extinção da doença. Segundo Guimarães e Bustamante (1954), o DDT possui, além de acção flebotomicida, tam-

bém valiosa acção repelente para este insecto, pois após a aplicação do DDT encontraram-se flebótomos bem junto dos domicílios e em número considerável, mas não dentro dos mesmos. Assim, em vilas ou patrimónios edificados após a derrubada das matas a aplicação do insecticida diminuirá em muito os casos de protozoose.

Segundo Floch (1957*a*), não só o DDT, mas o BHC e particularmente o dieldrin podem ser espalhados também no chão e ao redor das casas. Recomenda que estes insecticidas sejam espargidos num raio de 250 m ao redor das habitações, desde que os flebótomos têm voo curto.

Quanto à profilaxia individual dos trabalhadores e derrubadores de matas, Floch (1957*b*) recomenda a administração de 4 mg de pentamidina por quilo de peso; diz este autor que tal profilaxia foi usada, com bons efeitos aparentes, em algumas pessoas expostas à infecção durante várias semanas. Suas observações são, porém, muito escassas. Sugere o autor em apreço que se experimente a profilaxia por meio de uma dose semanal de uma 4-aminoquinolina, que tem certa eficiência no tratamento da «espundia».

Pessoa e col. (1946) recomendam a vacinação preventiva, principalmente para a protecção dos derrubadores de mata. A vacinação por meio de injecções de leptomonas mortas, na dose de 100 a 200 milhões destes organismos por centímetro cúbico, aumentando a resistência do homem à inoculação do germe, concorrerá para diminuir o número de casos da doença, que ocorrem durante sòmente alguns meses do ano epidémico (Coutinho, 1954).

## RESUMO

O A. estuda a distribuição geográfica da L. T. A. e mostra que esta parasitose tem sido assinalada em quase todo o hemisfério ocidental, desde o México até o Norte da República Argentina. Não é doença autóctone no Canadá, Estados Unidos, Uruguai e Chile.

Após considerações sobre a etiologia, a reprodução e a cultura da *Leishmania brasiliensis*, faz longas considerações sobre a epidemiologia da leishmaniose tegumentar americana. Mostra que a endemia predomina nas zonas húmidas florestais, não sendo observada em regiões áridas e secas, a menos que em determinadas épocas do ano se registrem microclimas favoráveis ao ciclo biológico dos insectos vectores. Também ataca a protozoose qualquer idade, sexo e raça. Os autores, em geral,

admitem ser a L. T. A. transmitida nas condições naturais por insectos do género *Phlebotomus*. No estado de São Paulo foram encontradas as seguintes espécies naturalmente parasitadas: *P. migonei*, 0,21 %; *P. withmani*, 0,20 %; *P. pessoai*, 0,29 %; *P. intermedius*, 0,14 %. A prova crucial, porém, da transmissão da *L. brasiliensis* pela picada do flebótomo ainda não foi obtida. A hipótese, todavia, da transmissão pelo flebótomo explica a epidemiologia da doença entre nós. Assim, as espécies encontradas, até hoje, infectadas têm hábitos silváticos ou semi-domésticos. No Brasil a moléstia não é encontrada nas cidades e vilas distantes das zonas florestais. No entanto, condições microclimáticas favoráveis podem determinar o aparecimento de casos nas vizinhanças de cidades. Pouco se sabe sobre a questão dos reservatórios vertebrados extra-humanos da *L. brasiliensis*. Foram descritos o cão e, mais recentemente, ratos silvestres como reservatórios do parasita.

Em seguida o A. estuda a patologia da doença, descrevendo o período de incubação, as lesões iniciais e as principais formas clínicas. Analisa a questão das lesões das mucosas e a sua frequência. Após descrever sumàriamente a anatomia patológica das lesões, analisa o diagnóstico clínico e os métodos de laboratório, tanto os directos (demonstração do parasita) como os indirectos (reacções sorológicas e alérgicas).

Após sumariar o tratamento, termina o seu estudo com a profilaxia, mostrando que o DDT veio pràticamente resolver este problema. Acha que no caso de trabalhadores da mata também pode usar-se a vacinação preventiva com leptomonas mortas.

## BIBLIOGRAFIA

Citamos a bibliografia mais recente sobre a leishmaniose tegumentar americana. Quanto à bibliografia até 1946, reportamos o leitor à monografia de PESSOA, S. B., e BARRETO, M. P. — *Leishmaniose Tegumentar Americana*. 1 vol., 527 pp., Publ. do Ministério da Educação e Saúde, Rio de Janeiro, 1948.

Também León publicou recentemente uma monografia sobre o assunto com extensa bibliografia: LEÓN, L. A. — *Leishmanias y Leishmaniasis (especialmente en América)*. 1957. Quito. Ed. Universitaria. (Cit. in *Trop. Diseases Bulletin*, **55** (3): 261, 1958.)

AZULAY, R. D. — *Leishmaniose Tegumentar*. Tese de livre-docência. Rio de Janeiro, 1 vol., 168 pp., 1952.

BARROS, R. P. DE, LIMA, M. L. M. T., e CORREIA, A. — «Leishmaniose nasal», *Rev. Hosp. das Clínicas*, **7**: 145-150, São Paulo, 1952.

BARRIENTOS, L. P. — «Um caso atípico de leishmaniose cutaneomucosa (espundia)», *Mem. Inst. Oswaldo Cruz*, **46** (2): 415-418, 1948.

BIAGI, F. — «Algunos comentarios sobre las leishmaniasis y sus agentes etiológicos. *Leishmania tropica mexicana*, nueva subespecie», *Medicina*, **32** (684): 435-438, México, Setembro, 10, 1953.

BIAGI, F. — *La Leishmaniasis Tegumentaria Mexicana y algunos Datos Médico-Estadísticos de Escórcega, Camp.* Tese do México, 1 vol., 119 pp., 1953a.

Biagi, F. — «Sintesis de 70 historias clínicas de leishmaniasis tegumentaria en México (úlcera de los chicleros)», *Medicina*, **32** (682): 385-396, México, Agosto, 25, 1953*b*.

Convit, J., e Lapenta, P. — «Sobre un caso de leishmaniasis tegumentaria de forma disseminada», *Rev. Policlínica de Caracas*, **18** (100): 153-158, 1948.

Convit, J., Reyes, O., e Kerdel, F. — «Disseminated anergic leishmaniosis. Report of three cases of a type clinically resembling lepromatous leprosy», *Arch. Dermat.*, **76** (2): 213-217, 1957.

Carrera Cobos, T. — «Anotaciones sobre la leishmaniosis selvática americana cutáneo-mucosa», *Rev. Ecuatoriana de Entom. y Parasit. Guayaquil*, **1** (2): 76-90, 1953.

Coutinho, J. O. — «Observações sobre a vacinação preventiva com leptomonas mortas na leishmaniose espontânea da cobaia: *Leishmania enriettii*», *Folia Clinica et Biologica S. Paulo*, **21** (3): 321-326, 1954.

Del Ponte, E. — «Complejos patogénicos de enfermedades disseminadas por artrópodes», *Anales de Med. Pública*, **2** 431-472, 1950.

Floch, H., e Casile, M. — «Intradermoreaction de Montenegro à la leishmanine», *Bul. Soc. Path. Exot.*, **48** (5): 636-642, 1955.

Floch, H. — «Épidémiologie de la leishmaniose forestière américaine en Guyane française», *Riv. di Malariologia*, **36** (4-6): 233-242, 1957.

Floch, H. — «Sur la prophylaxie de la leishmaniose forestière américaine», *Riv. di Malariologia*, **36** (4-6): 243-253, 1957.

Forattini, O. P., e Santos, M. R. — «Nota sobre a infecção natural de *Phlebotomus intermedius*, por formas em leptomonas, em foco de leishmaniose tegumentar americana», *Arq. Hig. e Saúde Púb.*, **17**: 171-174, S. Paulo, 1952.

Forattini, O. P. — *Algumas Observações sobre a Biologia de Flebótomos em Região da Bacia do Rio Paraná (Brasil)*. Tese de livre-docência, 1 vol., S. Paulo, 1954.

Forattini, O. P. — «Um foco de leishmaniose tegumentar na zona sul de São Paulo, Brasil», *Arq. Fac. Hig. e Saúde Púb. S. Paulo*, **11** (1): 23-24, 1957.

Forattini, O. P., Pattoli, D., e Aun, J. R. — «Algumas observações sobre o comportamento da *Leishmania brasiliensis* em cães», *Arq. Fac. Hig. e Saúde Púb. S. Paulo*, **7** (2): 137-155, 1953.

Forattini, O. R., Santos, M. R., e Pattoli, D. — «Nota sobre o encontro de leishmânias em roedores silvestres de zona endémica de leishmaniose tegumentar no estado de São Paulo, Brasil», Associação Paulista de Medicina, sessão de 5 de Maio de 1958, 1958.

Guimarães, F. N. — «Visceralização da *Leishmania brasiliensis* Viana, 1911, em *hamsters (Cricetus cricetus)*», *Brasil Médico*, **41**: 395-396, 1947.

Guimarães, F. N. — «Leishmaniose experimental», *O Hospital*, **40**: 25-46, 153-162 e 655-675, 1951.

Guimarães, F. N., e Bustamante, F. M. — «A aplicação domiciliária do DDT com base na profilaxia das leishmanioses», *Rev. Bras. de Malariol. e Doenças Trop.*, **6**: 127-130, Rio de Janeiro, 1954.

GUIMARÃES, F. N. — «Estudo de um foco de leishmaniose mucocutânea na Baixada Fluminense (estado do Rio de Janeiro)», *Mem. Inst. Osw. Cruz,* **53**: 1-11, 1955.

HERRER, A. — «Nota preliminar sobre leishmaniosis natural en perros», *Rev. Med. Experimental,* **7** (1-4): 62-66, 1948.

HERRER, A. — «Estudos sobre leishmaniosis tegumentaria en el Perú. IV — Observaciones epidemiológicas sobre la uta», *Rev. Med. Exp.,* Lima, **8**: 45-88, 1951*a*.

HERRER, A. — «Estudios sobre leishmaniosis tegumentaria en el Perú. V — Leishmaniosis natural en perros procedentes de localidades utógenas», *Rev. Med. Exp.,* Lima, **8**: 87-778, 1951*b*.

HERRER, A. — «Repercursión del uso casero de los insecticidas en la incidencia de la leishmaniosis tegumentaria del perro», *Rev. Med. Exp.,* Lima, **10** (2): 139-145, 1956.

HERRER, A., e BATTISTINI, M. G. — «Estudios sobre leishmaniosis tegumentaria en Perú», *Rev. Med. Exp.,* **8**: 9-119, 1951.

HERTIG, M., e FAIRCHILD, G. B. — «The control of *Phlebotomus* in Peru with DDT», *Am. J. Trop. Med.,* **28**: 207-230, 1948.

HERTIG, M., FAIRCHILD, G. B., e JOHNSON, C. M. — «Leishmaniasis transmission», *Gorgas Mem. Lab. An. Rep.,* 1956, pp. 9-11, 1957.

HOARE, C. A. — «Cutaneous leishmaniasis. Critical review of recent Russian work», *Trop. Dis. Bull.,* **41**: 331-345, 1944.

JAFFÉ, L. — «Nasal leishmaniasis americana in Panama», *Arch. Otolaryngology,* **60**: 601-611, 1954.

MOHIUDDIN, A. — «An investigation into the relationships between the species (parasite in man) of the genus *Leishmania* Ross, 1903, Part I», *Indian J. Med. Res.,* **40**: 7-23. «Part II», *Indian J. Med. Res.,* **40**: 171-192, 1952.

ORSINI, O. — «Aspectos epidemiológicos e clínicos da leishmaniose tegumentar americana no estado de Minas Gerais», *I Reunião Anual dos Dermossifil. Brasileiros,* pp. 11-26, 1945.

PEREIRA F.°, M. J. — «Casos de leishmaniose tegumentar no R. G. do Sul. Algumas considerações sobre o diagnóstico clínico-laboratorial. Tratamentos aconselháveis», *An. Fac. Med. Porto Alegre,* pp. 103-128, 1949.

PESSOA, S. B. — «Leishmaniasis, cutaneous, mucocutaneous and visceral, with special reference to its occurrence in the Americas», in GRADWOHL, SOTO e FELSENFED — *Clinical Trop. Medicine,* 1 vol. The L. V. Mosby C°, St. Louis, 1951.

PESSOA, S. B., e ROTBERG, A. — «Nota sobre o tratamento intensivo da leishmaniose tegumentar pelo tartarato de sódio e antimonila», *O Hospital,* **32**: 25-34, 1947.

PIFANO, C. F. — «La leishmaniasis tegumentaria en Venezuela», *Arch. Venezol. Patol. Trop. y Parasit. Med.,* **1**: 170-182, 1949.

PIFANO, F., MEDINA, R., FEBRES, M. M., e ROMER; M. — «La leishmaniasis tegumentaria americana», *Rev. de Sanid. y Assist. Soc.,* Caracas, **19**: 403-423, 1954.

PUPO, J. A. — «Estudo clínico da leishmaniose tegumentar americana», *Rev. do Hosp. das Clínicas,* **1**: 113-164, São Paulo, 1946.

PUPO, J. A. — «Contribuição para o estudo da leishmaniose tegumentar americana», *II Congr. Latino-Americano de Otorrinolaringologia e Broncoesofagia,* pp. 476-500, 1951.

RABELO, F. E., PORTUGAL, H., SERRA, O., e ROCHA, G. L. — «Leishmaniose tegumentar: formas clínicas, alergia específica, estrutura histológica e número de gérmens», *I Reunião Anual dos Dermossifil. Brasileiros,* pp. 37-71, 1945.

RAMOS E SILVA, J. — «Leishmanides. Considerações sobre a forma generalizada da leishmaniose tegumentar americana», *An. Bras. Dermat. e Sif.,* **25**: 125, 1950.

ROMAÑA, C., NAJERA, L., CONEJOS, M., e ABALOS, J. W. — «Leishmaniosis tegumentaria en perros de Tucuman», *An. del Inst. Med. Regional,* **2**: 283-292, 1949.

ROTBERB, A. — «Contribuição para o estudo da alergia na leishmaniose tegumentar americana», tese de livre-docência, *Rev. Hosp. N. S. Aparecida,* **5**: 3-83, 1952.

SALES, D. DE — «Geografia médica do Ceará. Distribuição geográfica da leishmaniose», *Rev. Bras. Med.,* **9**: 496-498, 1952.

SCHAFER, J. — «Recientes progresos en el tratamiento de la leishmaniose», *El Dia Médico,* **17**: 702-709, 1949.

# ASPECTOS CLÍNICOS DO CALAZAR AMERICANO

JOAQUIM EDUARDO DE ALENCAR [1]

## CASOS OBSERVADOS

O calazar foi diagnosticado pela primeira vez na América, em 1913, por Migone, no Paraguai, num doente procedente de Mato Grosso (Brasil) e após foram diagnosticados casos em diversos países (México, Guatemala, Salvador, Guiana Holandesa, Venezuela, Colômbia, Peru, Bolívia, Paraguai e Argentina (quadro 1).

No Brasil, até 1934, foram diagnosticados 41 casos, publicados no trabalho de Pena com os resultados das viscerotomias em cadáveres suspeitos de febre-amarela. Essas pesquisas, continuadas por Pará, mostraram 330 casos (quadro 2), demonstrando a existência de uma endemia no Nordeste e Leste brasileiros.

Até hoje foram diagnosticados *in vivo* 1874 casos nesses focos, havendo uma incidência particularmente grande no estado do Ceará (quadro 3, mapas 1 a 3). Aqui há variações anuais e regionais ligadas, tal como na Índia (Sen Gupta, 1951), aos infortúnios sociais.

## SINTOMAS APRESENTADOS

Trabalhando para a avaliação epidemiológica e para a organização do programa de profilaxia, tivemos contacto com a maioria dos casos observados e recebemos também regularmente as fichas clínicas de muitos dos restantes. Pudemos então obter informações preciosas

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que nos permitiram organizar o quadro da sintomatologia da moléstia americana.

QUADRO 1

| Países | Ano | Número de casos | Autores |
|---|---|---|---|
| México | 1953 | 1 | Villaseñor, Ruiloba, Rojas, Trevino e Campillo. |
| Guatemala | 1949 | 1 | Cabrera e León. |
| Salvador | 1950 | 1 | Bloch e Guillen. |
| Guiana Holandesa | 1954 | 1 | Winckel e Aalstein (*in* Deane, 1956). |
| Venezuela | 1941 | 1 | Martinez e Pons. |
| | 1942 | 1 | Potenza e Anduze. |
| | 1945 | 1 | Franco Palacios. |
| | 1946 | 1 | Misla Peña, Ron Pedrique e Hernandez (*in* Deane, 1956). |
| | 1950 | 1 | Potenza e Andola (*in* Deane, 1956). |
| | 1954 | 1 | Pifano (*in* Deane, 1956). |
| Colômbia | 1944 | 1 | Gast Galvis e Renjifo. |
| Peru | 1938 | 3 | Ciotola. |
| Bolívia | 1939 | 1 | Gatti, Boggino e Pietro. |
| | 1942 | 1 | Barros e Rosenfeld. |
| | 1949 | 1 | Arruda, Costa, Nahas e Rosenfeld. |
| Paraguai | 1945 | 1 | Boggino e Naas. |
| Argentina | 1926 | 2 | Mazza e Carnejo. |
| | 1934 | 1 | Inda, Vivoli e Vacarezza. |
| | 1936 | 2 | Romana, Torros e Klappenbach. |
| | 1941 | 1 | Fernandez Ithurrat. |
| | 1942 | 1 | Vivoli (*in* Deane, 1956). |
| | 1947 | 1 | Fonso e Ink. |

Esses sintomas colocam a doença dentro do esquema da endemia mediterrânea, mas alguns casos se assemelhavam aos observados na Índia e outros dão lugar a pensar-se na existência de dois tipos da moléstia no Brasil.

**Idade dos doentes**

A incidência se faz em 81,2 % no grupo de idades de 0 a 9 anos, o que a aproxima da doença mediterrânea. O quadro 4 e o gráfico 1 mostram essa aproximação com a Itália num extremo e o Sul da Índia no outro (quadro 5 e gráfico 2).

QUADRO 2 — *Casos de calazar verificados no Brasil por viscerotomia — 1932-1937 (Pena e Pará)*

| Estados | Anos | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 1932-35 | 1936-40 | 1941-45 | 1946-50 | 1951-55 | 1956-57 | |
| Pará | 6 | 8 | 3 | 1 | — | — | 18 |
| Maranhão | — | — | 1 | — | — | — | 1 |
| Piauí | 3 | — | 9 | 2 | — | — | 14 |
| Ceará | 34 | 41 | 39 | 35 | 3 | 1 | 153 |
| Rio Grande do Norte | 1 | 3 | 4 | 2 | — | — | 10 |
| Paraíba | — | — | 2 | — | — | — | 2 |
| Pernambuco | 3 | 2 | 3 | — | — | — | 8 |
| Alagoas | 5 | — | — | 8 | — | — | 13 |
| Sergipe | 11 | 9 | 5 | 1 | — | — | 26 |
| Baía | 15 | 8 | 30 | 16 | 10 | — | 79 |
| Minas Gerais | — | — | 1 | — | — | 1 | 2 |
| Mato Grosso | — | — | 2 | 1 | — | — | 3 |
| Goiás | — | — | — | — | — | 1 | 1 |
| *Total* | *78* | *71* | *99* | *66* | *13* | *3* | *330* |

QUADRO 3 — *Casos de leishmaniose visceral humana descritos no Brasil, nos diversos estados — 1913-1957*

| Estados | Anos | | Total |
|---|---|---|---|
| | 1913-52 | 1953-57 | |
| Pará | 8 | — | 8 |
| Piauí | — | 101 | 101 |
| Ceará | 7 | 1 493 | 1 500 |
| Rio Grande do Norte | — | 11 | 11 |
| Paraíba | 1 | 2 | 3 |
| Pernambuco | 4 | 1 | 5 |
| Sergipe | 1 | 1 | 2 |
| Baía | 8 | 189 | 197 |
| Minas Gerais | 1 | 42 | 3 |
| Mato Grosso | 4 * | — | 4 |
| *Total* | *34* | *1 840* | *1 874* |

* 1 destes casos foi importado de zona endêmica da Europa.

QUADRO 4 — *Calazar humano no Ceará. Incidência por idade — 1953-1956*

| Idades | Percentagem |
|---|---|
| — 1 ano | 8,54 |
| 1 ano | 4,13 |
| 2 anos | 23,17 |
| 3 anos | 16,34 |
| 4 anos | 8,29 |
| 5 a 9 anos | 20,24 |
| 10 a 14 anos | 6,50 |
| 15 a 19 anos | 3,90 |
| 20 a 29 anos | 3,90 |
| 30 a 39 anos | 2,30 |
| 40 anos e mais | 1,40 |

QUADRO 5 — *Calazar humano no mundo. Incidência por idade de 0 a 9 anos — 1924-1941*

| País ou região | Percentagem | Autor |
|---|---|---|
| Sicília (Itália) | 98,8 | Adler e Theodor. |
| Grécia | 96,0 | Bertrand. |
| Tunes (Tunísia) | 95,4 | Anderson. |
| Noroeste da China | 95,0 | Ho *et al.* |
| Sul da França | 89,7 | Giraud. |
| Ásia Central | 87,0 | Guerschenowitsch. |
| Ásia Central | 85,7 | Artamonoff. |
| Ceará (Brasil) | 81,1 | Alencar e cols. |
| Marselha (França) | 80,6 | Giraud, Ranque e Cabassu. |
| Piauí (Brasil) | 74,5 | Alencar e cols. |
| China | 63,0 | Chung *et al.* |
| Sul da Índia | 42,00 | Bertrand. |
| China | 40,1 | Scovel. |
| Bengala (Índia) | 37,0 | Bertrand. |
| China | 33,0 | Patton e Hindle. |
| Assan (Índia) | 30,8 | Bertrand. |
| Calcutta (Índia) | 21,2 | Bertrand. |
| Sul da Índia | 15,4 | Ksishman. |

Mapa 1 — Distribuição geográfica da leishmaniose visceral

**Período de incubação**

Há muitas dificuldades para estabelecer o período de incubação. Sendo uma moléstia de incidência rural, raramente urbana, desenvolve-se entre as populações de regiões tropicais sem a cultura suficiente para anotar pormenores ou mesmo fornecer informações precisas. Acresce mais que nas regiões afectadas há doenças que podem ser confundidas com o calazar.

Mapa 2 — Distribuição da leishmaniose visceral no Brasil

Dentre os casos observados, em um pôde ser apurado um período de 20 dias, pois os primeiros sintomas surgiram 20 dias após o nascimento (Alencar e Aragão, 1955), e em outro 16 meses. Devem ter os casos, na sua maioria, 2 a 5 meses de incubação.

**Período inicial**

As mesmas dificuldades notamos para estabelecer os sintomas do período inicial, pois os doentes são observados quase sempre no período de estado. O início é brusco ou gradual quanto à forma e

Mapa 3 — Municípios com calazar no estado do Ceará

de tipo entérico ou respiratório quanto aos sintomas. Os quadros 6, 7 e 8 mostram que realmente há uma frequência muito grande de sintomas respiratórios, sendo a tosse um dos dominantes (85,3 %), e de sintomas digestivos, como a diarreia, que aparecem também com alta

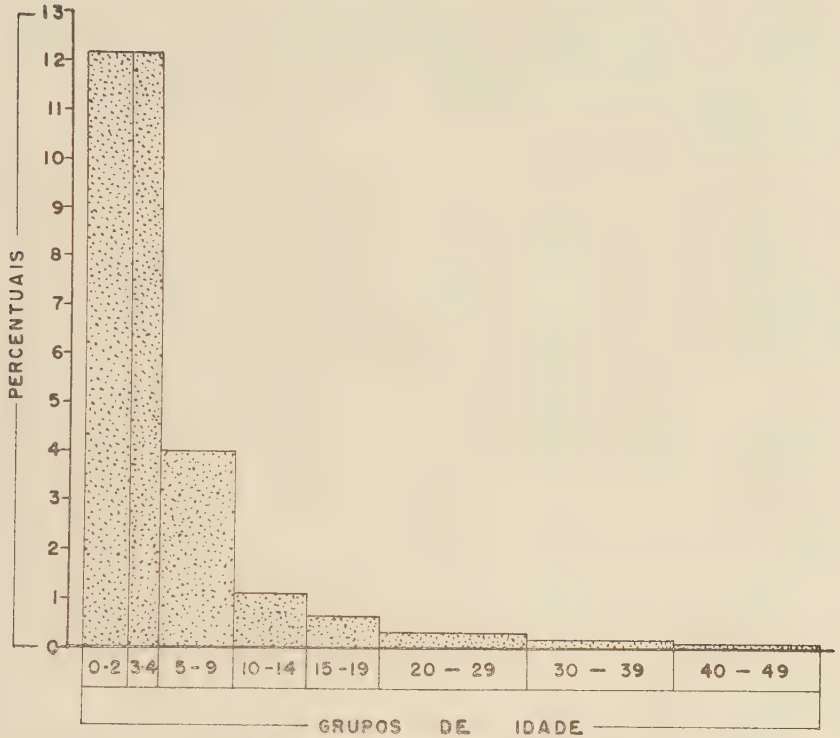


Gráfico 1 — Calazar humano no Ceará (1953–1956): incidência por idades

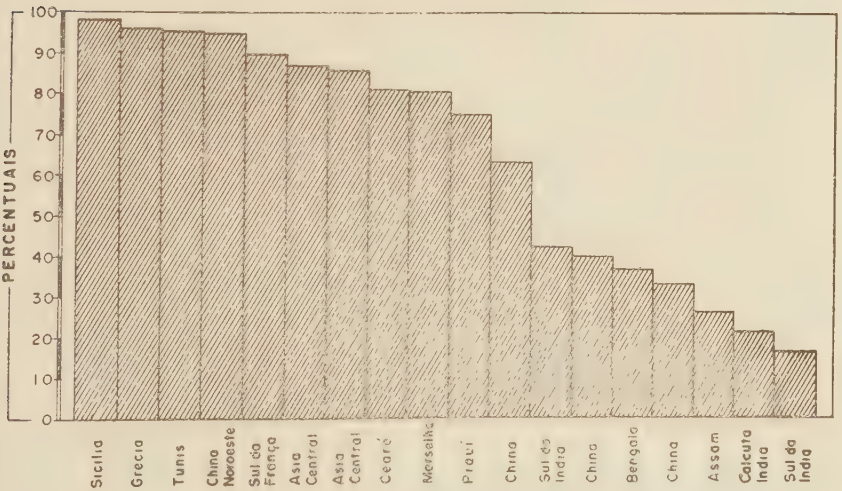


Gráfico 2 — Calazar humano: incidência no grupo de idades 0–9 anos em diversas regiões do mundo (tabela n.o 5)

frequência, caracterizando o quadro em que ocorrem outros sintomas (Alencar e Aragão, 1955; Prata, 1957; Rodrigues da Silva, 1957).

Nesse período a febre sempre existe, com emagrecimento, palidez e perda de apetite (96,1 %, 100 % e 67,9 %).

**Período de estado**

Esse período é mais rico de ensinamentos, pois nele se encontra a maioria dos casos. No Brasil as descrições clínicas assinalam sintomas que são comuns a outras regiões do mundo.

Os 1038 casos observados no Nordeste apresentaram os seguintes sintomas e sinais reais frequentes (quadros 6, 7 e 8): febre, esplenomegalia, hepatomegalia, micropoliadenia, anemia, tosse, diarreia, pulso rápido, suores, icterícia, edemas, depilação, etc.

Febre.

A febre é de carácter irregular, intermitente-remitente, com duas e três ascensões diárias (gráfico 3), considerada característica por Napier e Manson-Bahr.

A febre pouco frequentemente se acompanha de calafrios e suores. Napier os refere apenas no tipo malárico, de início, tal como Chung. Veronesi e cols. referem uma recidiva com calafrios e sudorese, ao passo que Meira e cols. referem um caso com calafrios nos primeiros 4 meses, mas nunca seguidos de sudorese. Como o calazar se acompanha habitualmente de complicações, muito frequentemente respiratórias, o aparecimento, num determinado momento, de calafrios poderá ser traduzido por uma intercorrência desse tipo.

Dentre os casos de Veronesi e cols. notamos um que por ocasião da recidiva da febre se apresentou com calafrios e sudorese e outro de Versiani (1943) com esses sintomas e cefaleia. Já nos casos de Meira e cols. (1948), «durante 10 meses a febre era elevada, diária, aparecendo em horas certas, sendo precedida por arrepios de frio ou calafrios nos primeiros 4 meses, mas nunca seguida de sudorese». Dentre as 6 observações de Pondé e cols., em 4 há arrepios de frio e em 3 havia sudorese. Os 4 casos de Barros e Rosenfeld e Maciel apresentaram calafrios e sudorese.

QUADRO 6 — *Sintomatologia do calazar. Observações de casos do Ceará — 1953-1957*

| Especificações | Número de observações | Percentagem |
|---|---|---|
| TEMPERATURA: | | |
| 36,0 a 36,9 | 249 | 24,4 |
| 37,0 a 37,9 | 408 | 42,7 |
| 38,0 a 38,9 | 254 | 25,9 |
| 39,0 a 39,9 | 63 | 6,4 |
| 40,00 e mais | 6 | 0,6 |
| *Total de observações* | *980* | *100,0* |
| ASPECTO GERAL: | | |
| Bom | 79 | 7,9 |
| Regular | 660 | 65,6 |
| Precário | 197 | 19,6 |
| Ruim | 19 | 1,8 |
| Péssimo | 51 | 5,1 |
| *Total de observações* | *1 006* | *100,0* |
| AUMENTO DO BAÇO: | | |
| 0 | 22 | 2,2 |
| 1 | 57 | 5,6 |
| 2 | 163 | 16,2 |
| 3 | 282 | 28,1 |
| 4 | 315 | 31,4 |
| 5 | 166 | 16,5 |
| *Total de observações* | *1 005* | *100,0* |
| AUMENTO DO FÍGADO: | | |
| 0 cm a 2 cm | 369 | 38,1 |
| 3 cm a 4 cm | 406 | 42,0 |
| 5 cm a 6 cm | 126 | 13,0 |
| 7 cm e mais | 67 | 6,9 |
| *Total de observações* | *968* | *100,0* |
| NÚMERO DE PULSAÇÕES: | | |
| — 100 | 207 | 23,4 |
| 100 a 129 | 436 | 49,2 |
| 130 a 149 | 192 | 21,7 |
| 150 e mais | 51 | 5,7 |
| *Total de observações* | *886* | *100,0* |

QUADRO 7 — *Sintomas de calazar informados pelos pacientes no momento do exame — Ceará, 1953-1957*

| Sintoma | Vezes em que foi informado | |
|---|---|---|
| | Número | Percentagem |
| Emagrecimento | 969 | 96,1 |
| Epistaxe | 228 | 22,6 |
| Tosse | 860 | 85,3 |
| Apetite normal | 324 | 32,1 |
| Diarreia | 663 | 65,8 |
| Palidez | 1 008 | 100,0 |
| *Número de observações* | *1 008* | *100,0* |

QUADRO 8 — *Sintomas de calazar observados no momento e após o exame — Ceará, 1953-1957*

| Sintoma | Vezes em que foi informado | |
|---|---|---|
| | Número | Percentagem |
| Queda de cabelo | 470 | 45,3 |
| Edemas | 443 | 42,7 |
| Estomatite | 93 | 9,0 |
| Epistaxe | 117 | 11,3 |
| Bronquite | 761 | 73,3 |
| *Número de observações* | *1 038* | *100,0* |

ESPLENOMEGALIA.

Tal como assinalou Napier, na Índia, «o aumento do baço é o aspecto mais constante, mas não é incomum estar aparentemente sem aumento».

Em função do tempo de evolução da doença, medindo o aumento pela classificação de Hackett, vemos que muitas vezes o baço cresce

ràpidamente, de modo a atingir em 1 mês o grau 5, mas de um modo geral cresce regularmente com menor rapidez (Alencar e Aragão, 1955, quadro 9).

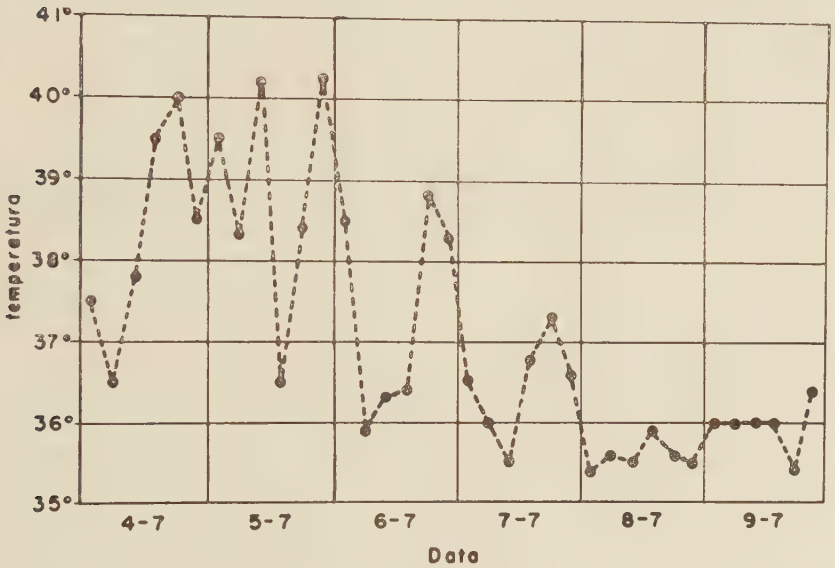


Gráfico 3 — Gráfico de temperatura do doente. Ficha n.o 157 (de 4 em 4 horas), 1954

QUADRO 9 — *Grau de esplenomegalia em função do tempo de evolução da moléstia*

| Grau de esplenomegalia (Hackett) | Tempo de evolução (em meses) | | |
|---|---|---|---|
| | Médio | Máximo | Mínimo |
| 1 | 0,8 | 1 | 0 |
| 2 | 1,8 | 4 | 1 |
| 3 | 3,1 | 9 | 1 |
| 4 | 3,9 | 12 | 1 |
| 5 | 4,9 | 12 | 1 |

Em resumo, o baço é de consistência mole, liso e indolor; torna-se doloroso quando há perisplenite (dor sempre difusa) ou quando há infarto (dor difusa, que logo se torna localizada). Os baços duros

correspondem quase sempre à concomitância de outra moléstia, como malária.

«Os baços de menor volume, abaixo do grau 3, foram apreciados apenas no estado do Ceará, facto que se deve atribuir à maior precocidade no diagnóstico dos casos naquele estado» (Rodrigues da Silva, 1957, quadro 6).

Hepatomegalia.

Alencar e Aragão (1955) em apenas 3 casos dentre 174 não encontraram aumento do fígado (97,7 %). Nos demais o aumento era de 1 cm a 10 cm abaixo do rebordo costal, medidos na linha mamilar. E nos casos em que o baço se apresentava normal o fígado foi encontrado aumentado. É a confirmação do que referiu Napier, pois o aumento do fígado era de 2 cm a 3 cm.

Todos os casos publicados por autores brasileiros mostraram esse aumento e entre nós a média é de 3 cm.

O fígado é de superfície lisa, o bordo fino, mole e raramente o encontramos discretamente doloroso.

Prata (1957) anotou sensibilidade dolorosa em 28,6 % dos casos. D'Oelsnitz diz que a hepatomegalia (na forma mediterrânea) existe quase sempre, mas aparece tardiamente e não adquire uma grande importância senão nas formas avançadas da infância e nas formas crónicas prolongadas do adulto. O aumento de volume do fígado pode excepcionalmente predominar e realizar uma forma hepatomegálica da doença, individualizada recentemente por Poinso (1932).

Também em alguns dos nossos casos a hepatomegalia foi notável (fig. 1), havendo ainda mais o meteorismo abdominal, ascite e emagrecimento do tórax, pernas e braços, dando ao doente um aspecto particular.

Hipertrofia dos gânglios.

Encontrámos em nossos casos 94,2 % de hipertrofia dos gânglios inguinais, epitrocleanos, axilares e cervicais (Alencar e Aragão, 1955; Prata, 1957); Rodrigues da Silva (1957) encontrou-os em 100 % dos casos. Mais dificilmente podemos asseverar que essas micropoliadenias correm exclusivamente por conta do calazar e não são devidas a outras causas. Chagas e cols. (1938) também as assinalaram.

Alterações hematológicas.

A anemia é constante no calazar americano e é levemente microcítica e hipocrómica (Alencar e Carneiro, 1956; Rodrigues da Silva, 1957). Num estudo em 123 casos encontrámos 8,9 % com menos de 2 milhões de hematias por milímetro cúbico de sangue, 47,9 % entre 2 e 3 milhões, 38,3 % entre 3 e 4 e apenas 4,9 % com mais de 4 milhões. Ao lado observámos a leucopenia, com neutropenia e plaquetopenia, mas no início de alguns deles, ou em complicações, houve em muitas oportunidades a leucocitose.

Alterações na pele e pêlos.

A característica pigmentação cutânea do calazar não tem sido referida no Brasil pelos diversos autores que tratam do assunto, e sim apenas um aumento de pigmentação em 4 %. Mas a palidez dos tegumentos é sinal precoce e é observada em todos os casos. Em 7 casos houve manchas de púrpura, dentre os quais um óbito.

A queda dos pêlos tem sido constatada por nós em 45,3 % dos casos (figs. 2 e 3). Mas o aspecto de cílios alongados, referido por Pittaluga como sinal característico, foi encontrado em 91,4 % dos casos (Alencar e Aragão, 1955).

Alterações do aparelho digestivo.

Aqui vamos encontrar como manifestação principal as diarreias (Alencar e Aragão, 1955, 48,3 %; Prata, 1957, 42,1 %; Rodrigues da Silva, 1957, 63 %). Já agora a percentagem é de 65,8 %.

As estomatites eram referidas em 9 % dos casos, mas não têm sido anotados casos mais graves, como *noma*. Há uma importância muito grande também na questão do apetite. Diz Chung que «o apetite é relativamente normal e a boa aparência da língua, usualmente, diferencia a moléstia de uma febre entérica». Mas entre nós 100 % dos casos têm língua saburrosa (Alencar e Aragão, 1955). Rodrigues da Silva (1957) liga esses aspectos a estados carenciais.

Fig. 1 — Doente: J. A. B. Idade: 6 anos. Residência: Maranguape, Ceará

Fig. 2 — Doente: M. P. I. Idade: 4 anos. Residência: Sítio Umari, município de Itapagé, Ce.

SINTOMAS DO APARELHO RESPIRATÓRIO.

Comummente, no calazar há uma associação de infecções do aparelho respiratório. «O sistema respiratório é preliminarmente inclinado aos processos inflamatórios». diz Napier (1946). Ao lado disso há uma tosse seca, irritante, desacompanhada de qualquer sinal

Fig. 3 — Doente': T. A. do E. S. Idade : 8 anos. Residência : Sítio Bananeira, município de Ubajara, Ce.

denunciador de uma associação; é uma tosse sem expectoração em 85,3 % dos nossos casos.

Com frequência notamos taquipneia em consequência do deslocamento pronunciado do parênquima pulmonar sob o efeito da elevação diafragmática condicionada principalmente pela hepatomegalia (Rodrigues da Silva, 1957).

Também podem ser ouvidos estertores crepitantes (Meira e cols., 1948) ou subcrepitantes (Versiani, 1943). Encontramos esses sinais nos muitos casos de bronquites (73,3 %) da nossa casuística.

Fig. 4 — Doente: F. E. P. Idade: 3 anos e 3 meses. Ficha 140-R. Peso: 7,5 kg. Baço: 5. Fígado: 0. Residência: José Nunes, Frade (Jaguaretama)

SINTOMAS DO APARELHO CIRCULATÓRIO.

As alterações do pulso são as mais características. O pulso, rápido, está quase sempre acima de 100 (Alencar e Aragão, 1955, 99,4 %; Prata, 1957, 90 %). No total das observações temos, no entanto, 76,6 %.

Rodrigues da Silva (1957), estudando alguns casos pelo electrocardiograma, assinalou que essa taquicardia é sinusal.

As bulhas são normais ou há sopros anémicos. A pressão arterial é baixa. A ascite é frequentemente observada. Em 1 caso houve descompensação cardíaca.

QUADRO 10 — *Proteínas do soro sanguíneo em casos de calazar*

| Data | Nome | Proteínas totais | Serina | Globulina | | | | Razão S/G |
|---|---|---|---|---|---|---|---|---|
| | | | | $\alpha 1$ | $\alpha 2$ | $\beta$ | $\lambda$ | |
| 21-3-54 | Simplício Silva Sobrinho . . . | 75,5 | 22,3 | 3,6 | 11,9 | 6,0 | 31,7 | 0,42 |
| 26-2-58 | Maria Lurdes Aragão . . . . | 61,0 | 13,6 | 2,7 | 5,7 | 5,6 | 33,4 | 0,28 |
| 13-2-58 | Manuel Messias . . . . . | 82,5 | 25,2 | 1,4 | 8,7 | ← | 47,3 | 0,44 |
| 7-3-58 | João Florentino da Silva . . . | 138,3 | 22,0 | 3,3 | 4,2 | 6,9 | 101,9 | 0,19 |
| 4-3-58 | Maria Serafina Nunes. . . . . | 108,0 | 28,6 | ← | 21,0 | ← | 58,4 | 0,36 |
| 26-2-58 | António Gonzaga . . . . . | 76,8 | 59,3 | ← | 9,1 | 3,9 | 44,5 | 0,33 |

QUADRO 11 — *Óbitos por calazar no estado do Ceará — 1953-1957*

| Causa imediata | Número de óbitos | Percentagem |
|---|---|---|
| Ignorada . . . . . | 21 | 44,7 |
| Broncopneumonia . . . . . | 9 | 19,2 |
| Anemia aguda . . . . . | 5 | 10,6 |
| Gastrenterite . . . . . | 2 | 4,3 |
| Toxicose . . . . . | 2 | 4,3 |
| Enterorragia . . . . . | 1 | 2,1 |
| Edema agudo do pulmão . . . . . | 1 | 2,1 |
| Hipertermia (após esplenectomia). . . . . | 1 | 2,1 |
| Anemia e broncopneumonia . . . . . | 1 | 2,1 |
| Anemia e nefrose. . . . . | 1 | 2,1 |
| Caquexia . . . . . | 1 | 2,1 |
| Insuficiência cardíaca . . . . . | 1 | 2,1 |
| *Total*. . . . . | *47* | *100,0* |

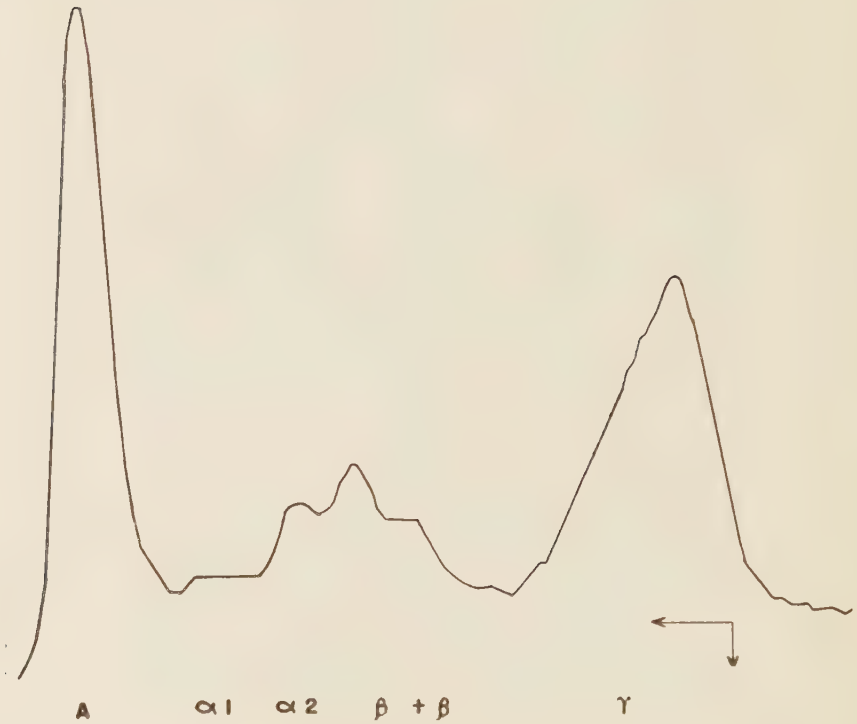


Gráfico 4 — Curva electroforética do soro sanguíneo (doente: S. S. S.)

Gráfico 5 — Curva electroforética do soro sanguíneo (doente: M. L. A.)

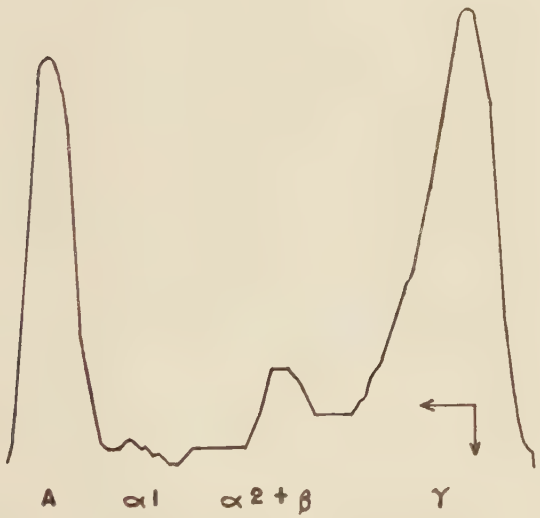


Gráfico 6 — Curva electroforética do soro sanguíneo (doente: M. M.)

Gráfico 7 — Curva electroforética do soro sanguíneo (doente: J. F. S.)

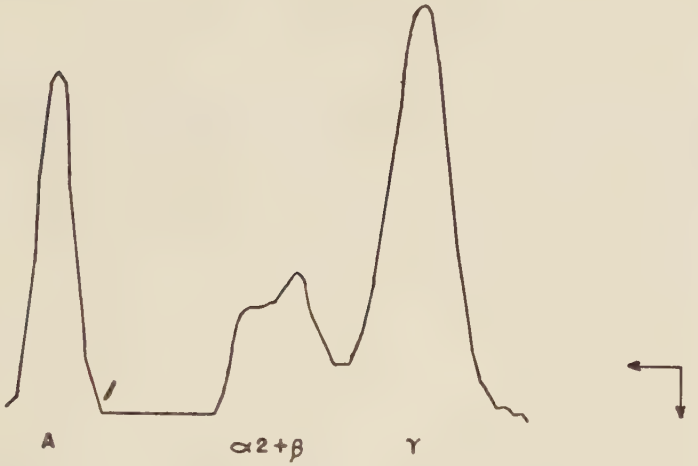


Gráfico 8 — Curva electroforética do soro sanguíneo (doente: M. S. N.)

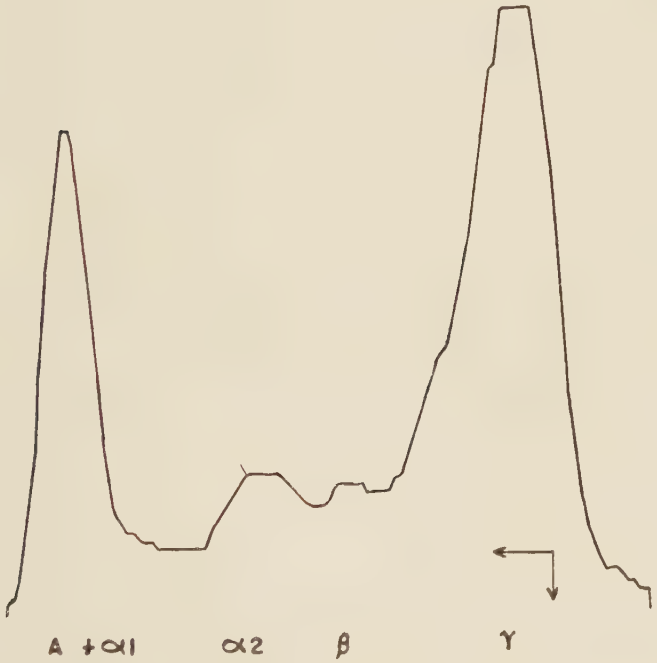


Gráfico 9 — Curva electroforética do soro sanguíneo (doente: A. G.)

Outras alterações.

*Disproteinemias.* — Há uma baixa das proteínas do soro sanguíneo com baixa da serina e inversão da razão serina/globulina, alteração tanto maior quanto mais considerável é o aumento da globulina gama (quadro 10 e gráficos 4 a 9). Em alguns casos em início há aumento das proteínas totais, mas a inversão da razão S/G permanece em cifras também muito baixas.

*Edemas.* — Observámos em nossos casos, em grande número de vezes, os *edemas* (27 %) (Alencar e Aragão, 1955). Os autores brasileiros comummente os assinalam (Veronesi e cols., 1954; Pondé e cols., 1942; Meira e cols., 1948).

Os edemas são habituais nos casos avançados, por conta, naturalmente, da desproteinemia e anemia.

*Icterícia.* — A icterícia não é comum no início da moléstia, mas no período de estado é bastante frequente, como sinal de uma hepatite leishmaniótica. Em nossos casos surgiu o sintoma acentuadamente apenas seis vezes, apesar de o soro ter sempre um índice ictérico elevado.

*Dores articulares.* — Alguns doentes referem também dores articulares.

**Período final**

A morte no calazar se dá no período final, muitas vezes por caquexia. Os nossos casos, quanto ao aspecto geral, mostram 5,1 % de péssimos que evoluem já para a caquexia (fig. 4).

No entanto, o óbito poderá dar-se por complicações, tais como broncopneumonias, pneumonias e hemorragias. Ocorrerá também por anemia aguda e insuficiência hepática quando surgem condições cirróticas (Rodrigues da Silva, 1957). A mortalidade entre nós é de 4,5 %, mas metade dos óbitos (44,7 %) ocorrem quando o doente está afastado do serviço e se dão por causa ignorada; provàvelmente disenterias bacilares (quadro 11).

## TIPO CLÍNICO E TIPO EPIDEMIOLÓGICO

O calazar indiano tem manifestações viscerais frequentemente intestinais que no tipo mediterrâneo são raras (Fraga de Azevedo, 1954). Entre nós essas manifestações são frequentes na infância. No mais nos filiamos ao tipo mediterrâneo, numa posição mais central na escala. Aqui, na infância, há maior número de manifestações purpúricas e edematosas e maior anemia (Rodrigues da Silva, 1957).


## RESUMO

O A. apresenta uma análise dos sintomas ocorridos em mais de mil casos de calazar observados no Nordeste brasileiro, especialmente no Ceará.

Ficou evidenciado que a incidência se faz tal como no Mediterrâneo, em maior quota na infância, especialmente de 0 a 4 anos de idade, com sintomas semelhantes aos encontrados naquela área do mundo.

Os sintomas iniciais são: febre, esplenomegalia, hepatomegalia, micropoliadenia, emagrecimento, anemia, além de sintomas ligados aos aparelhos respiratório, digestivo e circulatório, tais como tosse, diarreias e taquicardia. A febre é do tipo remitente-intermitente, com duas ou três ascensões diárias.

Houve uma influência regional, referente às condições de alimentação da região ou às condições epidemiológicas em geral, sobre esses sintomas.

No período de estado vemos acentuados esses sintomas e a ocorrência de complicações, das quais as mais comuns são a pneumonia, as disenterias, as gastrenterites e as estomatites. Não têm sido observadas, no entanto, infecções mais graves, como *noma*.

As repercussões hematológicas são frequentes, produzindo uma anemia acentuada; há leucopenia, com neutropenia e linfocitose. Em poucos casos, ocorridos no período epidémico, manchas purpúricas.

Há acentuada gama-globulinemia, com inversão da razão serina/globulina.

As repercussões cutâneas são mínimas, com uma escassa distribuição de leishmânias na pele e raros casos de lesões.



## SUMMARY

The Author presents a report of the symptoms existing in over a thousand cases of kalazar observed in northeastern Brazil, mainly in the state of Ceará.

It has been clearly demonstrated that the incidence recurring just as it did in the Mediterranean, in greater portion in infancy, chiefly in people aged from 0 to 4 years, hand symptoms similar to those found in that area of the world.

The initial symptoms are: fever enlargement of liver, spleen and lymphatic glands, emaciation, anaemia, beside the symptoms linked with the respiratory, digestive and circulatory organs, such as coughing, diarrhea and tachycardia. The fever is of the remittent-intermittent kind, having two or three risings a day.

There has been a regional influence upon these symptoms regarding the conditions of food in the country, or the epidemiologic conditions in general.

In the later stages we find these symptoms to be very marked and also the occurrence of implications, of which the commonest are: pneumonia, dysentery, gastroenteritis and stomatitis.

More serious infections, however, like *cancrum oris,* have not been noticed.

Hematologic repercussions are frequent, producing a noticeable anaemia. There is leucopenia with decrease of granulocytes and rise of lymphocytes.

Purple speckles are scarcely found in the epidemic period.

There is a marked increase of gama-globulin with inversion of the serin/globulin ratio.

The cutaneous repercussions are too rare, with a scarce distribution of leishmanias on the skin and unfrequent cases of lesions.

## BIBLIOGRAFIA


Adler, S., & Theodor, O. — «Mediterranean kala-azar. Introduction and epidemiology», *Proc. Roy. Soc., London, ser. B.* **108**: 447-453, 1931.

Albuquerque, A. F. R., Brito, S., e Morais, M. O. — *Importante Foco de Mal de Chagas e Leishmaniose Visceral Americana no Vale do Cariri (Estados do Ceará e Pernambuco).* Separata de *O Hospital,* **21**: 61-69, 1942.

Alencar, J. E. de — *Leishmaniose Visceral no Novo Mundo.* XII Congresso Brasileiro de Higiene, 1955. Separata de *Publicações Médicas,* **27**: 196, 1956.

Alencar, J. E. de, e Aragão, T. C. — *Tratamento da Leishmaniose Visceral em Sobral, Ceará.* XII Congresso Brasileiro de Higiene. *Publicações Médicas,* **26**: 193, 1955.

Alencar, J. E. de, e Aragão, T. C. — *Leishmaniose Visceral no Ceará. Sintomas observados em 174 casos.* XII Congresso Brasileiro de Higiene. *Publicações Médicas,* **28**: 197, 1955.

Alencar, J. E. de, Cantidio, W. M., e Cavalcante, D. N. — *Calazar em Fortaleza.* XIII Congresso Brasileiro de Higiene. Fortaleza, Ceará, Brasil, 1956.

Alencar, J. E. de, Dinis, J. O., Pessoa Filho, F. P., Aragão, T. C., e Correia, T. A. — *Alguns focos de calazar no Piauí.* XIII Congresso Brasileiro de Higiene. Fortaleza, Ceará, Brasil, 1956.

Alencar, J. E. de, Holanda, D., e Cavalcante, J. D. N. — «Calazar no vale do Jaguaribe», *Rev. Bras. Malariologia,* **8** (1): 33-47, 1956.

Alencar, J. E. de, e Carneiro, P. A. M. — *Índices eritrocitários no diagnóstico do calazar.* XIII Congresso Brasileiro de Higiene. Fortaleza, Ceará, Brasil, 1956.

ALENCAR, J. S. DE e NETO, B. C. — *Leishmaniose canina no Ceará.* XIII Congresso Brasileiro de Higiene. Fortaleza, Ceará, Brasil, 1956.

ALENCAR, J. E. DE, e SHERLOCK, L. A. — *Fauna flebotómica do Ceará.* XIII Congresso Brasileiro de Higiene. Fortaleza, Ceará, Brasil, 1956.

ALMEIDA, E. — «Leishmaniose visceral. Primeiro caso no estado da Paraíba», *Bras. Méd.,* **59**: 82-83, 1945.

ANDERSON, C. — «Chronique du kala-azar en Tunisie», *Arch. Inst. Pasteur de Tunis,* **23**: 455-564, 1934.

ARRUDA, W., COSTA, S. C., NAHAS, S., e ROSENFELD, G. — «Leishmaniose visceral americana», *Brasil Méd.,* **63**: 64-65, 1949.

ARTAMANOF, A. S. — «Contribution à l'étude épidémiologique de la leishmaniose viscérale de l'Asie Centrale», *Russian Jl. Trop. Med.* **7** (4): 205-217, 1929.

AZEVEDO, J. F. — *L'état actuel du problème du kala-azar au Portugal,* **32** (3): 234-254, 1954.

BARROS, O. M., e MACIEL, P. — «Leishmaniose visceral americana. Dois casos de um novo foco na Baía», *Rev. Clín. S. Paulo,* **26**: 50-55, 1951.

BARROS, O. M., e ROSENFELD, G. — «Leishmaniose visceral americana. Um caso na Bolívia», *Rev. Clín. S. Paulo,* **11**: 91-99, 1942.

BARROS, O. M., e ROSENFELD, G. — «Leishmaniose visceral americana. Novo caso», *Rev. Clín. S. Paulo,* **15**: 97-102, 1944.

BERTRAND, M. — *Kala-azar Méditerranéen chez l'adulte.* Tese de Alger, 1931.

BLOCH, M., y GUILLEN, G. — «Kala-azar en El Salvador», *Arch. Col. Med. El Salvador,* **3**: 90-96, 1950.

BOGGINO, J., y HAAS, L. C. — «Primer caso ciertamente autoctone de leishmaniasis visceral», *An. Fac. Ciencias Méd. Univ. Nac. Asunción,* **5**: 319-324, 1945.

CABRERA, M. A., y LEÓN, R. — «Historia del primer caso clínico de leishmaniasis visceral descubierto en Guatemala», *Public. Inst. Investig. Científ. Guatemala,* n.º 2. Res. in *Trop. Dis. Bull.,* **48**: 349, 1949.

CANÇADO, J. R., GAMA, G., MOURÃO, O. G., BRENNER, Z., ANTONACCIO, F., LANA, G. C., e ROMEIRO, B. — «Calazar autóctone de Minas Gerais», *O Hospital,* **50** (3): 373-392, 1956.

CARVALHO, J., e PESSOA, S. B. — «Dois novos casos de leishmaniose visceral na Paraíba», *An. Fac. Med. Paraíba,* vol. I (em publicação), 1955.

CHAGAS, E., CASTRO, G. O., e FERREIRA, L. C. — «Leishmaniose visceral americana. IX — Considerações sobre a leishmaniose visceral americana (nova entidade mórbida do homem na América do Sul)», *Mem. Inst. Oswaldo Cruz,* **32**: 381-389, 1937.

CHAGAS, E., FERREIRA, L. C., DEANE, G., DEANE, L., e GUIMARÃES, N. — «Leishmaniose visceral americana. II — Estudo epidemiológico», *Mem. Inst. Oswaldo Cruz,* **33**: 138-206, 1938.

CHUNG, H. L., and FENG, L. CH. — «A résumé of kala-azar work in China», *Chinese Medical Journal,* **71**: 421-464, 1953.

COUTINHO, A., e LIRA, A. — «Leishmaniose visceral em Pernambuco. Considerações clínicas e hematológicas sobre um novo caso», *Rev. Bras. Med.,* **11** (2): 89-97, 1954.

DEANE, L. M. — *Leishmaniose Visceral no Brasil. — Estudos sobre reservatórios e transmissores realizados no estado do Ceará.* S. N. E. S. Rio de Janeiro, 1956.

DEANE, L. M., e DEANE, G. — «Estudos sobre a leishmaniose visceral americana», *O Hospital,* **2**: 191-202, 1937.

DEANE, L. M., e DEANE, M. P. — «Encontro de cães naturalmente infectados por *Leishmania donovani* no Ceará», *O Hospital,* **45**: (6): 703-707, 1954.

DEANE, L. M., e DEANE, M. P. — «Encontro de leishmânias nas vísceras e na pele de uma raposa, em zona endémica de calazar, nos arredores de Sobral, Ceará», *O Hospital,* **45**: (4): 419-421, 1954.

DEANE, L. M., e DEANE, M. P. — «Leishmaniose visceral urbana (no cão e no homem) em Sobral, Ceará», *O Hospital,* **47**: 75-87, 1955.

DEANE, L. M., e DEANE, M. P. — «Observações preliminares sobre a importância comparativa do homem, do cão e da raposa *(Lycalopex vetulus)* como reservatórios da *Leishmania donovani* em área endémica de calazar no Ceará», *O Hospital,* **48**: 61-67, 1955*b*.

DEANE, L. M., DEANE, M. P., e ALENCAR, J. E. DE — «Efeito imediato da dedetização domiciliária sobre a densidade de flebótomos em focos endémicos de calazar no Ceará». Apres. na sessão de 4 de Novembro Dep. Hig. & Med. Trop. Assoc. Paulista Med. Resumo in *Rev. Paulista Med.,* **46**: 129-130, 1955.

DEANE, M. P., e DEANE, L. M. — «Infecção natural do *Phlebotomus longipalpis* por leptomonas, provàvelmente de *Leishmania donovani,* em um foco de calazar no Ceará», *O Hospital,* **55** (6): 697-702, 1954.

DEANE, M. P., e DEANE, L. M. — «Considerações sobre a transmissão da leishmaniose visceral no Ceará», *O Hospital,* **48**: 347-364.

D'OELSNITZ, M. — «Kala-azar in children and adults; review of recent clinical acquisitions», *Pres. Med.,* **44**: 28-30, 1936.

FERNANDEZ ITHURRAT — 1941 — *In* VIVOLI, 1942, *apud* DEANE, 1956.

FIGUEIREDO, A. — «Leishmaniose visceral no Brasil», *J. de Medicina de Pernambuco,* **32** (4): 50-56, 1936.

FONSO, C., e INK, J. — *Rev. Med. & Cien. Af.,* 36, 1947. Resumo in *Bol. Of. San. Panam.,* **27**: 40, 1948.

FRANCO PALACIOS, M. — «Kala-azar infantil en el Estado de Carobobo», *Rev. Polic!. Caracas,* **14**: 526-531, 1945.

GAST GALVIS, A., y RENGIFO, S. — «Leishmaniasis visceral. Estudio Epidemiológico del primer caso diagnosticado en Colombia», *An. de la Soc. Biol. de Bogotá,* **1**: 161-168, 1944.

GATTI, O., GOGGINO, J., et PRIETO, C. — «Un nouveau foyer de leishmaniose viscérale en Amérique du Sud», *Bull. Soc. Path. Exot.,* **32** (6): 602-605, 1939.

GIRAUD, P., RANQUE, J., et CABASSU, H. — «Épidémiologie de la leishmaniose viscérale humaine méditerranéenne, en particulier dans ses rapports avec la leishmaniose canine», *Arch. Franc. Pediat.,* **11**: 337-353, 1954.

GUERSCHENOWITSCH, R. — «Le kala-azar infantile en Moyenne Asie», *Presse Méd.,* **21**: 403-405, 1936.

GUIMARÃES, F. N., e BUSTAMANTE, F. M. — «A aplicação domiciliária de DDT como base de profilaxia da leishmaniose. Estudo de um foco de leishmaniose

mucocutânea cinco anos depois da aspersão periódica com aquele insecticida», *Rev. Bras. Malariologia & D. Trop.*, **6**: 127-130, 1954.

INDA, VIVOLI y VACCAREZA — «Estudio anatomo-clínico de un caso de kala-azar en el adulto (fiebre negra). Primera observación en nuestro país», *La Semana Médica*, Buenos Aires, **41** (6): 413-424, 1934.

MACIEL, P., e MONTEIRO, G. — *Leishmaniose visceral americana (2 casos de um novo foco na Baía)*. Separata da *Revista Clínica de São Paulo*, **27** (5-6): 55-60, 1951.

MACIEL, P., e ROSENFELD, G. — «Leishmaniose visceral americana. Um novo caso de um novo foco», *Rev. Clín. S. Paulo*, **21**: 51-61, 1947.

MANSON-BAHR, P. — *Tropical Diseases*. 3rd ed., pp. 142-163, 1930.

MARTINEZ, N. A., y PONS, A. R. — «Primer caso de kala-azar en Venezuela». *Gac. Med. Caracas*, **48**: 329-332, 1941.

MARTINS, A. V., BRENER, Z., MOURÃO, O. G., LIMA, M. M., SOUSA, M. A., e DA SILVA, J. E. — *Calazar autóctone em Minas Gerais*. XIII Congresso Brasileiro de Higiene. Fortaleza, Ceará, Brasil, 1956.

MAZZA, S., y CARNEJO, A. J. — «Primeros casos autóctones de kala-azar infantil comprovados en el Norte de la República», *Bol. Inst. Clín. Quir.*, **11**: 140-144, 1926.

MEIRA, J. A., JAMRA, M. A., e LIMA, M. L. M. T. — «Leishmaniose visceral americana. Considerações clínicas, hematológicas e anátomo-patológicas a propósito de um caso com mielose aplástica», *Arq. Fac. Hig. S. Pub. Univ. S. Paulo*, **2**: 253-300, 1948.

MIGONE, L. E. — «Un cas de kala-azar à Assunción (Paraguay)», *Bull. Soc. Path. Exot.*, **6**: 118-120, 1913.

NAPIER, L. E. — *The Principles and Practice of Tropical Medicine*. The Mac Millian Company, New York, pp. 135-177, 1946.

OLIVEIRA, A. C. — «Um caso de leishmaniose visceral americana», *O Hospital*, **13** (3): 465-470, 1938.

PARÁ, MADUREIRA — «Viscerotomia hepática», *Argus Medical*, n.os 5 e 6, 1955.

PENA, H. A. — «Leishmaniose visceral no Brasil», *Bras. méd.*, **46**: 949-950, 1934.

PESSOA, S. B., DA SILVA, L. H. P., e FIGUEIREDO, J. — *Calazar endémico em Jacobina (estado da Baía). Aprés.* XII Congresso Brasileiro de Higiene. Belém, Pará, 1955.

POINSON, R. — «Hepatic form of infantile kalazar; 2 cases», *Presse Méd.*, **39**: 1920-1922, 1931.

PONDÉ, R., MANGABEIRA FILHO, O., e JANSEN, G. — «Alguns dados sobre a leishmaniose visceral americana e doença de Chagas no Nordeste brasileiro», *Memórias do I. O. C.*, 37-3, 1942.

POTENZA, L., y ANDUZE, J. — «Kala-azar en el Estado do Bolivar, Venezuela», *Rev. Policl. Caracas*, **11**: 312-317, 1942.

PRATA, A. — *Estudo Clínico e Laboratorial do Calazar*. Tese de concurso, Salvador, Baía, 1957.

RODRIGUES DA SILVA, J. — *Leishmaniose Visceral (Calazar)*. Tese de concurso, Faculdade Nacional de Medicina da Universidade do Brasil, 1957.

ROMANA, C., TORRES, A., y KLAPPENBACH, E. — «Hallazgo de un foco de kala-azar infantil en el Chaco (nota prévia)», *La Semana Médica,* Buenos Aires, **43** (10): 954, 1936.

SCOVEL, F. G. — «Kala-azar; a review of its incidence and epidemiology in China and clinical observations on 585 cases», *Ann. Inst. Med.,* **21**: 607-627, 1944.

SEN GUPTA, P. C. — «A report on kala-azar in Assam», *Indian Med. Gaz.,* **86** (6-7): 266-271, 312-317, 1951.

TAVARES, A., e FONTE, J. — «Sobre o 1.° caso de leishmaniose visceral com verificação parasitológica em Pernambuco», *Laboratório clínico (R. J.),* **22** (174): 133, 1942.

VERONESI, R. — «Leishmaniose visceral (calazar)», *Rev. de Med.,* **39**: 20, 1955.

VERONESI, R., CASTRO, R. M., MARQUES, J. C., FIORILLO, A. M., ZUCOLLOTO, M., CZAPSKI, J., SALLES, H. L. B., e AMATO NETO, V. — «Leishmaniose visceral (calazar) no Brasil. Estudo do quadro clínico e humoral de 15 novos casos», *Rev. Hosp. Clín. S. Paulo,* **10**: 86-111, 1955.

VERONESI, R., JAMRA, M., SILVA, O. R. S., CRUZ, O., e FIORILLO, A. — «Leishmaniose visceral (calazar). Estudo do quadro clínico hematológico e electroforético», *Rev. Hosp. Clín. S. Paulo,* **9**: 13-50, 1954.

VERSIANI, O. — «Leishmaniose visceral americana», *Brasil Médico,* **25-26**: 268-271, 1943.

VILLASEÑOR, J. B., RUILOBA, J., ROJAS, B., TREVIÑO, A., y CAMPILLO, S. C. — «Un caso de kala-azar en Méjico», *Bol. Of. Sanit. Panamer.,* **34**: 23-30, 1953.

# CONSIDERACIONES SOBRE LA EPIDEMIOLOGÍA DEL KALA-AZAR EN LA AMÉRICA NEOTROPICAL [1]

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## CONSIDERACIONES GENERALES

En Guatemala, A. C., recientemente se han registrado dos casos de leishmaniosis visceral (kala-azar), uno el año de 1949 (1), el otro en abril de este año (2), ambos con diagnóstico clínico y parasitológico completo. Es notorio que los dos casos pertenecen al mismo Departamento de El Progreso, que se desarrollaron en zonas casi desérticas, vale decir, epidemiológicamente homólogas, con una similitud panorámica sorprendente, con un foco endémico de kala-azar descrito de Minas Gerais por A. Viana Martins y col. (3).

También es evidente que estos dos casos se incubaron en sitios absolutamente alejados de toda contaminación exterior; es decir, cuyo orígen no podría atribuirse a reservorios humanos importados, lo cual vendría a revalidar el nombre de *L. chagasi* de los parasitólogos sudamericanos para el agente etiológico del kala-azar americano, en tanto pudiera hallarse un medio de diferenciación específica para todas las leishmanias conocidas del hombre. En un terreno de tal pobreza biológica como este foco endémico de kala-azar en Guatemala, es notoria también la escaséz de una fauna de vertebrados a

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[1] Este trabajo fué hecho bajo los auspicios y con el equipo del Instituto de Investigaciones Científicas, Universidad de San Carlos de Guatemala.

la cual pudiera incriminarse como reservorios naturales de esta *Leishmania*. No hace muchos años se describió en los cobayos (4) la *L. enrietti*, que aunque no parece trasmisible al hombre, mantiene, sí, la fundada conjetura del orígen animal de las leishmaniosis, sobre todo en lugares, como los aquí descritos, que no han tenido ningún contacto con emigrantes infectados, abriendo así la puerta a la investigación de cuales pudiesen ser aqui esos reservorios naturales del kala-azar guatemalense.

Los flebótomos en nuestro país, aún no han salvado la etapa monte-vivienda humana para elegir sus criaderos, pero sí, en las encuestas realizadas a esta zona endémica, pudo comprobarse plenamente la invasión de la vivienda por los flebótomos adultos, mientras estos dípteros no discriminan para sus hábitos hematófagos entre los distintos vertebrados y así alguna vez pudieran hacer contacto con un animal reservorio, explicándose por este hecho el exíguo número de los flebótomos, que por esta eventualidad adquieren la infestación con leishmanias trasmisibles al hombre.

## ENCUESTAS EPIDEMIOLÓGICAS

La primera y segunda encuestas fueron objeto y argumento de otros trabajos ya publicados (1-2), la segunda se llevó a cabo entre el 7 y el 11 de febrero de este año, cuyos resultados revistieron tal importancia, que estimularon la tercera, objeto del presente trabajo. En efecto, se reveló por primera vez en Guatemala la invasión de la vivienda humana por los flebótomos, que igualmente pudieron ser capturados en un gallinero, y, finalmente, que un estudio entomológico permitió establecer que esta especie invasora era el *Phl. longipalpis*, ya incriminado como vector del kala-azar en Sudamérica y del cual se ha dicho que «há sempre *Phl. longipalpis* em densidade apreciável em qualquer época do ano quando surgem casos de calazar humano e canino» (5), y en el mismo sentido se pronuncian Forattini y dos Santos (6). No se halló, sin embargo, ningún caso humano de *L. visceral* fuera del que motivó aquí la expedición ni la presencia de perros leishmaniosos.

La tercera encuesta se verificó del 17 al 21 de marzo de este año, hacia caseríos vecinos a El Jicarillo, orígen del 2.º caso de kala-

azar guatemalense, es decir, La Laguna, Puente Barranquillo, Agua Dulce y el Poxt, llevando como finalidad, 1.º la observación de eventuales casos de kala-azar, 2.º la pesquisa de perros enflaquecidos y con lesiones de dermatosis facial y 3.º la pesquisa de flebótomos.

Todas estas aldeas guardan similitud topográfica, estando cercanamente a los 700 m de altitud sobre el nivel del mar, a relativa corta distancia de dos ríos caudalosos, el río Motagua y su afluente el río Plátanos, de clima cálido, escasa vegetación, con montes muy pedregosos, formando frecuentes anfractuosidades o grietas posiblemente propicias al desarrollo de flebótomos.

En esos semidesolados parajes se cultiva maíz y cereales en pequeña escala que apenas procuran la supervivencia de sus escasos moradores, todos mestizos, descendientes de los colonizadores españoles.

La totalidad de las viviendas humanas de estas aldeas son de bahareque, adobe y caña, los techos de teja o palma y pisos de tierra; teniendo una o dos habitaciones a lo sumo, donde con frecuencia los animales domésticos: cerdos, perros, aves de corral, etc., pernoctan con las personas.

Ninguna casa posee servicio sanitario.

La precipitación pluvial anual en estos lugares es escasa, a tal grado que los habitantes sufren frecuentemente pérdidas en sus cosechas.

Durante la estación seca el agua para consumo la obtienen de pequeños «ojos de agua», situados a cientos de metros del centro de la población, acarreándola las mujeres en tinajas y en bestias.

Aldea La Laguna, altura 700 m sobre el nivel del mar:

| | |
|---|---|
| Habitaciones humanas . . . . . | 31 |
| Personas adultas . . . . . | 70 |
| Niños . . . . . | 96 |

Aldea Agua Dulce, altura 600 m sobre el nivel del mar:

| | |
|---|---|
| Habitaciones humanas . . . . . | 16 |
| Personas adultas . . . . . | 48 |
| Niños . . . . . | 43 |

Pronto hubo de descartarse la presencia de casos de kala-azar, forzosamente esporádicos por el escaso número de habitantes, y cabe presumir que también por el muy escaso número de flebótomos infestados con leishmanias.

El segundo objetivo, o sea la investigación de perros leishmaniosos, fué lo mismo, negativa.

La tercera encuesta fué la pesquisa de flebótomos, positiva en La Laguna y Puente Barranquillo, pero de captura extradomiciliar, efectuada en gallineros.

Aquí debe hacerse notar que en Guatemala es evidente el descenso en la densidad de los flebótomos durante la época seca, acentuándose este descenso en marzo y abril, al menos en las especies selváticas (en Guatemala existen más de una veintena de especies de flebótomos, actualmente en estudio, para la publicación de una monografía del autor).

Por otra parte, los flebótomos son bien conocidos por los habitantes de esta región, en donde, por razón de su densidad, tuvieron que notar su presencia y denominar como «jejenes» a falta de otro nombre vernáculo, aunque equívoco por designación (culicoides). Una futura encuesta permitirá comprobar si en realidad la densidad de los flebótomos en estos lugares es como refieren los habitantes, que llegan a provocar humaredas para librarse de sus picaduras, invadiéndolos en los campos y en sus propias viviendas.

## LOS FLEBÓTOMOS

Aparte de dos especies aún no identificadas en esta región, la especie predominante es *Phl. longipalpis* y, al parecer, la única que invade la vivienda humana y en su proximidad inmediata, los gallineros.

Las capturas de flebótomos efectuadas en proximidad de esta región desde el año 1948, casi con solo un interés entomológico para la publicación de una monografía nacional sobre estos dípteros, nos permiten ahora, a la vista del protocolo respectivo, la revelación curiosa de que el *Phl. longipalpis* solo ha sido capturado en el Departamento de El Progreso, a cuya jurisdicción pertenecen los tres primeros casos de kala-azar descritos en Guatemala, y solo una captura

de esta especie en proximidad del pueblo de Teculután, Departamento de Zacapa, de entre un número de 51 localidades pertenecientes a 9 Departamentos de la República.

En poseción ahora de este indispensable factor epidemiológico, se hizo evidente que, dadas las condiciones topográficas de esta región cuyo estudio epidemiológico es objeto de este trabajo, permite hacer dos consideraciones de importancia:

1.° Que para un vector tan pequeño y somáticamente débil y de vuelo de corta distancia, halló sin embargo vías de dispersión adecuadas a sus pobres condiciones biológicas. Así puede con facilidad trazarse sus vías de dispersión através de las barrancas interpuestas entre un sistema de elevaciones del escabroso terreno allí reinante, cuyas cimas solo exhiben vegetación nula o escasa. Por el contrario, la profundidad de las barrancas se halla cubierta por una cadena de árboles, en mayoría mangos *(Mangifera indica)*, que propician la formación de criaderos de los flebótomos;
2.° La dispersión de la especie a través de tal cadena casi ininterrumpida de árboles. Esto es lo que se ha comprobado desde El Conacaste hasta El Puente Barranquillo de Norte a Sur. y viceversa, desde El Conacaste a Teculután, del Departamento de Zacapa, para la especie *Phl. longipalpis* y otras especies aún no identificadas.

En resúmen, tres casos de kala-azar en esta zona de Guatemala, en sítios ecológicamente homólogos para la especie *Phl. longipalpis*, reconocida como trasmisora de la leishamaniosis visceral en la América Neotropical, permiten trazar ya una área epidemiológica potencial de kala-azar por la suma de factores positivos:

1.° Existencia de tres casos comprobados, presumiblemente ocurrencia de otros desapercibidos o no diagnosticados correctamente;
2.° Las condiciones geográficas y topográficas. La correlación de zonas epidemio-ecológicamente homólogas;

3.° La presencia del agente trasmisor de la enfermedad a través de Sud y Centro América. Falta aún un eslabón en la cadena biológica de transmisión de kala-azar nostras, el reservorio natural de esta *Leishmania,* hecho que abre la puerta a futuras investigaciones para descubrirlo.

## RESUMEN

En Guatemala, de 1949 a 1957, se llegó al diagnóstico de dos casos de kala-azar en un departamento nacional de similitud panorámico y ecológicamente análoga con focos endémicos del Brasil.

Estos dos casos se incubaron en sitios alejados de toda contaminación exterior.

Se pudo comprobar la invasión de la vivienda por flebótomos adultos de la especie *Phl. longipalpis,* ya incriminado en Sudamérica como transmisor exclusivo en casos de leishmaniosis visceral. La tercera encuesta epidemiológica se verificó en el vecindario geográfico del segundo caso de kala-azar guatemalense.

Las localidades objeto de la encuesta se encuentran entre 600 m y 700 m de altura sobre el nivel del mar y en la vecindad de ríos caudalosos.

Se dá cuenta de tres aldeas visitadas, con detalles de altura y censo de población.

Se descartó la presencia de casos actuales de kala-azar. Igualmente investigación de perros leishmaniosos fué negativa.

La pesquisa de flebótomos y su estudio entomológico reveló que el *Phl. longipalpis,* con exclusividad, se hallaba invadiendo la vivienda humana. El estudio del protocolo entomológico del Instituto de Investigaciones Científicas permitió establecer que casi exclusivamente el Departamento de El Progreso cuenta entre su fauna con el *Phl. longipalpis.*

Los dos casos de kala-azar en esta zona de Guatemala se desarrollaron en sitios ecológicamente homólogos con los descritos en la América Tropical (Brasil) y Neotropical; concepto que permite trazer ya un área epidemiológicamente potencial del kala-azar por la suma de factores positivos: primero, en el caso de Guatemala, existencia de dos casos humanos comprobados; segundo, por la correlación de zonas ecológicamente homólogas a las descritas en Sudamérica; y tercero, por la presencia del *Phl. longipalpis.*

Queda por investigar un eslabón en la cadena epidemiológica del kala-azar nostras; es decir, el reservorio natural de esta *Leishmania.*

## CONCLUSIONES

1.° En Guatemala se ha hecho el diagnóstico clínico y parasitológico de tres casos de kala-azar en zonas de similitud epidemiológica con un foco endémico descrito de Minas Gerais, Brasil.

2.° Ambos casos autóctonos evolucionaron en sitios alejados de toda contaminación exterior; es decir, sin contacto con emigrantes de cualquier tipo.

3.° Aquí se ha comprobado la invasión de la vivienda y su vecindad inmediata por el *Phl. longipalpis,* ya incriminado en Sudamérica como vector aparentemente exclusivo del kala-azar.

4.° La encuesta en esta área endémica tuvo por finalidad de orientación:

*a*) Observación de eventuales casos de kala-azar;

*b*) La pesquisa de perros infectados;

*c*) La pesquisa de flebótomos. La pesquisa comprendió tres caseríos, sin hallar nuevos casos de kala-azar ni perros infectados y sí, en todos, la presencia del *Phlebotomus longipalpis.*

5.° Ya puede trazarse en Guatemala un área epidemiológicamente potencial de kala-azar:

*a*) Por existencia de casos comprobados;

*b*) Por la correlación de zonas epidemio-ecológicamente homólogas con las descritas en el Brasil;

*c*) Por la presencia del agente transmisor reconocido: el *Phlebotomus longipalpis.* Futuras investigaciones determinarían el eslabón faltante en la cadena biológica de transmisión del kala-azar nostras.

## BIBLIOGRAFÍA

1. Cabrera, Marco Antonio — «Historia del primer caso clínico de leishmaniosis visceral (kala-azar) descubierto en Guatemala», *Publ. Inst. Invest. Científ. Guatemala,* 1949.
2. Figueroa G., Luís Noé — *Leishmaniosis visceral (kala-azar), segundo caso clínico descubierto en Guatemala.* Imprensa Universitaria, Guatemala, A. C., 1958.
3. Viana Martins, A., e Brener, T., y col. — *Rev. Brasileira de Malariologia e Doenças Trop.,* Rio de Janeiro, Brasil, Outubro de 1946.
4. Adler & Halff, L., obs. on L. Enrietti Muñiz & Medina — *An. Trop. Med. & Parasit.,* **49**: 37, 1948.
5. Alencar, J. E., e col. — «Calazar no vale do Jaguaribe, Ceará», *Rev. Brasil. de Mal. e Doenças Trop.,* Rio de Janeiro, 1956.
6. Forattini, P. Oswaldo, e dos Santos, Moacir — «Nota sobre um foco de leishmaniose tegumentar americana no estado de Mato Grosso, Brasil», *Rev. Brasileira da Malariologia e Doenças Trop.,* Rio de Janeiro, Brasil, 1956.

# EPIDEMIOLOGIA E PROFILAXIA DO CALAZAR AMERICANO [1]

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No continente americano a leishmaniose visceral ocorre, como infecção autóctone, em grande parte da Região Neotropical, sendo que os limites conhecidos de sua incidência correspondem às latitudes de cerca de 19° N. e 29° S. Já foi assinalada no México, Guatemala, Salvador, Venezuela, Colômbia, Bolívia, Paraguai, Argentina, Brasil e, possìvelmente, Guiana Holandesa.

Até recentemente, em vista do pequeno número de casos encontrados, a moléstia era tida como esporádica nas Américas. Hoje sabe-se que, pelo menos em parte do Brasil, ela incide endemo-epidèmicamente, constituindo em certas áreas do Nordeste do País um problema de saúde pública.

Até o fim do ano passado, a julgar pelas publicações a que tivemos acesso, foram registrados no continente 2175 casos de calazar, sendo 1838 diagnosticados em vida e 337 *post mortem;* a grande maioria desses casos — 2145 — foi encontrada no Brasil (quadros 1 e 2).

Não temos entretanto suficiente base para avaliar a prevalência do calazar no continente, nem mesmo no Brasil, onde a moléstia tem sido mais estudada. Os números acima citados não podem constituir-se em índices dessa prevalência: dos pacientes observados em vida poucos foram encontrados através de inquéritos epidemiológicos, a maioria tendo sido descoberta ao procurarem os doentes ou seus pais, espontâneamente, o tratamento, em poucos postos especializados

[1] Presented *in absentia*.

existentes em alguns estados; em vista dos minguados recursos das populações mais atingidas, julgamos que o número de casos que procura assistência representa apenas pequena fracção daqueles que realmente ocorrem. A maioria dos médicos do interior não está alertada para a presença dessa doença: no Brasil, por exemplo, até 1952, apenas 33 casos clínicos foram descritos; os demais 1782 foram achados a partir de 1953, quando os médicos de algumas das áreas endémicas aprenderam a reconhecer a doença [1]. Convém frisar ainda que neste mesmo país foram diagnosticados 330 casos pelo exame de amostras de fígado obtidas *post mortem*, para pesquisa de lesões de febre-amarela, dentre cerca de 470 000 óbitos (93, 95).

No Brasil o calazar incide em todos os estados litorâneos, desde o Pará até à Baía, e nos estados centrais de Minas Gerais, Goiás e Mato Grosso; aparentemente poupa os estados sulinos. Os maiores focos ficam no Nordeste do País, sendo os estados do Ceará, Piauí e Baía os mais atingidos (quadro 2).

Fora do Brasil o número de doentes verificado ainda é muito pequeno (quadro 1). Na Venezuela, os 14 casos descritos até 1957 distribuíam-se pelos estados de Aragua, Bolivar, Carabobo, Cojedes, Guarico, Lara e Distrito Federal (66, 74, 80, 86, 97, 101, 102). Fomos recentemente informados, pelo Dr. J. Convit, do encontro de mais 3 casos nesse país, provenientes dos estados de Guarico e Zulia e do Distrito Federal.

Os 7 casos assinalados na Argentina provêm de Salta ou do Chaco (62, 65, 73, 82, 107, 116).

A literatura registra 3 pacientes como oriundos da Bolívia: 1 que teria contraído sua infecção no território de Yungas (68) e 2 residentes do lado boliviano da ferrovia Brasil-Bolívia (12, 15).

Dos 3 doentes diagnosticados no Paraguai (18, 68, 85) sòmente 1 (18) é seguramente autóctone.

Na Guatemala, até 1957, havia sido assinalado apenas 1 caso (21); porém este ano foi encontrado mais 1 (64), ambos de aldeias do departamento El Progreso.

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[1] Esses casos foram apresentados em numerosos estudos particularizados, correspondentes às referências bibliográficas n.os 1 a 6, 8 a 12, 14, 16, 22 a 24, 27, 33, 36, 44, 55, 78, 81, 83, 85, 92, 97, 100, 103, 108 a 110, 112 a 114.

QUADRO 1 — *Casos autóctones de leishmaniose visceral diagnosticados no continente americano, por país, em vida ou* post mortem, *até 1957*

| País | Número de casos | | |
|---|---|---|---|
| | Em vida | *Post mortem* | Total |
| México | 1 | — | 1 |
| Salvador | 1 | — | 1 |
| Guatemala | 1 | — | 1 |
| Guiana Holandesa | — | 1 | 1 * |
| Venezuela | 13 | 1 | 14 |
| Colômbia | — | 1 | 1 |
| Bolívia | 1 | 2 | 3 |
| Paraguai | — | 1 | 1 |
| Argentina | 6 | 1 | 7 |
| Brasil | 1 815 | 330 | 2 145 |
| *Total* | *1 838* | *337* | *2 175* |

* Provàvelmente autóctone.

QUADRO 2 — *Casos autóctones de leishmaniose visceral diagnosticados no Brasil, por estado, em vida ou* post mortem *até 1957*

| Estado | Número de casos | | |
|---|---|---|---|
| | Em vida | *Post mortem* | Total |
| Pará | 8 | 18 | 26 |
| Maranhão | — | 1 | 1 |
| Piauí | 101 | 14 | 115 |
| Ceará | 1 500 | 153 | 1 653 |
| Rio Grande do Norte | 11 | 10 | 21 |
| Paraíba | 4 | 3 | 7 |
| Pernambuco | 5 | 8 | 13 |
| Alagoas | — | 13 | 13 |
| Sergipe | 2 | 26 | 28 |
| Baía | 145 | 79 | 224 |
| Mato Grosso | 3 | 3 | 6 |
| Goiás | — | 1 | 1 |
| Minas Gerais | 34 | 2 | 36 |
| Ignorado | 2 | — | 2 |
| *Total* | *1 815* | *330* | *2 145* |

Outros países contam apenas com 1 doente cada: México, no estado de Guerrero (115), Salvador (17) e Colômbia (67). Um paciente diagnosticado na Guiana Holandesa é tido como provàvelmente autóctone (117). Os doentes descobertos nos Estados Unidos são sabidamente importados, excepto 1, contaminado acidentalmente em laboratório.

## EPIDEMIOLOGIA

Embora a transmissão do calazar tenha sido observada ou conseguida de várias maneiras, sem a interferência de um insecto vector, o seu mecanismo habitual é a picada de flebótomos infectados, conforme demonstrado na Índia, China e Mediterrâneo. Portanto, na cadeia epidemiológica da doença temos de considerar *a*) as fontes de infecção, *b*) os transmissores e *c*) a população susceptível, elementos esses que podem ser influenciados pelo *d*) meio ambiente.

Fontes de infecção. — Nos estudos pioneiros feitos sobre a epidemiologia do calazar no continente americano pela Comissão Evandro Chagas, cães foram encontrados espontâneamente parasitados no Brasil (32, 33). A Comissão entretanto não os julgava, nem ao homem, fontes primárias da infecção, acreditando no papel primordial de um animal silvestre, que não logrou encontrar.

Todavia, o papel do cão como um dos reservatórios na Região Neotropical é hoje facto geralmente aceito. Cães infectados têm sido achados em quase todos os focos brasileiros de calazar (3, 4, 6, 7, 9, 20, 34, 43, 47, 48, 75, 79, 91, 100), e o facto de não terem sido encontrados nos outros países onde casos humanos foram verificados talvez decorra da escassez de exames aí procedidos nos animais, pois sòmente em poucas oportunidades tem sido procurado o parasitismo em cachorros nos focos não brasileiros da doença: na Venezuela, em áreas do estado de Carabobo, onde haviam sido verificadas infecções humanas, e na Guatemala, em aldeia do departamento El Progreso, onde uma criança doente fora encontrada, foram examinados alguns cães, com resultado negativo (64, 99).

Em estudos recentes, efectuados no Nordeste brasileiro, foi verificado que, além do cão doméstico, um canídeo campestre muito comum na região, a «raposa» *Lycalopex vetulus,* se apresenta espon-

tâneamente parasitado (43, 46, 47, 56); seu papel epidemiológico será discutido após o estudo dos reservatórios domésticos.

A importância do homem e do cão como fontes de infecção no calazar americano tem sido comparada sob vários aspectos.

A capacidade infectante dos hospedeiros para o insecto transmissor foi avaliada indirectamente pela procura de leishmânias em trechos do seu organismo acessíveis ao transmissor — sangue e pele — e directamente, pela infecção experimental do vector. O parasitismo sanguíneo mostrou-se escasso em ambos os reservatórios. O parasitismo cutâneo foi verificado no Brasil em pacientes humanos não tratados, quer em lesões (49, 104, 112), quer na pele aparentemente sã (19, 43, 45, 60, 105). A leishmaniose dérmica pós-calazar, que em algumas áreas da Ásia é responsável pela importância do homem como fonte de infecção nos períodos interepidémicos, só foi assinalada no Brasil de forma inconteste uma vez (22). Nos cães doentes a frequência e a abundância do parasitismo cutâneo têm sido confirmadas em vários estudos feitos no Brasil (3, 4, 6, 7, 9, 20, 35, 37, 38, 39, 43, 47, 48, 49, 69, 77, 111). Observações comparativas efectuadas no estado do Ceará resultaram no encontro de leishmânias na pele obtida por biopsia em 16,3 % dos doentes humanos e 77,6 % dos cães; além disso, a intensidade do parasitismo cutâneo nos cães é, na maioria das vezes, muito maior que na pele humana (43, 49, 60).

A infecção experimental do transmissor *(Phlebotomus longipalpis)* foi conseguida com maior frequência e intensidade em cães do que em pessoas doentes. Em experiências efectuadas ainda no Brasil, 28,5 % dos pacientes humanos e 75 % dos cães infectaram os insectos. As infecções de flebótomos alimentados em cães foram, em geral, mais intensas do que as obtidas nos que sugaram pessoas; porém, em alguns destes também se observou multiplicação intensa de leptomonas, com bloqueio da faringe (43, 49).

Estas observações mostram que, individualmente, o cão é um reservatório mais importante, provàvelmente devido ao seu maior parasitismo cutâneo, mas que não se pode desprezar o homem como fonte de infecção.

Por outro lado, foi verificado que, em natureza, homem e cão são prontamente sugados pelo transmissor (43, 59).

A frequência comparativa da infecção desses hospedeiros nas áreas endémicas ainda não pôde ser convenientemente avaliada. Os

métodos usados para a descoberta dos casos humanos e caninos não são, evidentemente, os mesmos. Os doentes humanos têm sido encontrados entre indivíduos cujos sintomas os tornam suspeitos e nos quais se procura então fazer o diagnóstico etiológico, quase sempre através da punção esternal. A pesquisa de casos caninos tem de ser processada de modo bem diverso, pois, como é sabido, uma certa proporção de cães infectados não apresenta sintomas evidentes, aliás conforme tem sido confirmado no decorrer de estudos feitos no Brasil (4, 20, 43, 48, 91). O achado de cães parasitados tem decorrido do seu exame sistemático, quase sempre através da punção de fígado, biopsia de pele, ou ambos. Autores brasileiros têm-se manifestado sobre o valor comparativo do exame de esfregaços de fígado ou de pele no diagnóstico rotineiro da leishmaniose visceral canina em inquéritos epidemiológicos, opinando uns o emprego isolado da biopsia de pele, que seria método mais sensível e fácil de realizar (6, 7, 20, 77), julgando outros que os dois processos se equivalem e que, como a utilização simultânea de ambos revela um número maior de casos do que o emprego isolado de um só, será melhor critério examinar concomitantemente um fragmento de fígado e um de pele (4, 43, 47, 48). Embora sabidamente o baço e a medula óssea sejam, em geral, mais ricos em leishmânias que o fígado, a punção esplénica ou a óssea não têm sido usadas sistemàticamente nos inquéritos de leishmaniose canina, por serem mais difíceis de executar. É sabido, também, que no diagnóstico do calazar canino, como no humano, a pesquisa do agente etiológico nem sempre é positiva. Prova muito mais sensível — a reacção de fixação do complemento com antígeno de bacilos de tuberculose — tem sido recentemente empregada no Brasil (88, 89), onde a sua execução com a técnica quantitativa foi pela primeira vez efectuada e onde o seu valor no diagnóstico da leishmaniose visceral canina foi também, em primeira mão, comprovado (20, 90, 91). Num estudo realizado no estado do Ceará a punção hepática e biopsia de pele revelaram leishmânias apenas na metade dos animais cuja RFC foi positiva (91) e num foco de calazar do estado de Minas Gerais a diferença foi ainda maior (20). Essa reacção mostrou-se precoce, pois foi positiva em casos que, na ocasião, não apresentavam sintomas e em que a pesquisa de leishmânias fora negativa e nos quais foram encontrados parasitas em exames posteriores (20, 91). Parece ser também relativamente específica para fins práticos, mostrando-se nega-

tiva em cães de áreas não endémicas (20, 90, 91); porém, pensamos que este aspecto merece mais estudos, pois entre indivíduos humanos a reacção é positiva (se bem que em títulos de diluição mais baixos) em doentes de leishmaniose tegumentar, moléstia de Chagas, lepra e tuberculose (89). Entretanto a RFC não é ainda utilizada rotineiramente nos inquéritos epidemiológicos de leishmaniose visceral no Brasil, o descobrimento dos casos sendo feito, conforme vimos, pelo exame parasitológico.

Não obstante a imperfeição dos métodos de diagnóstico utilizados, os resultados de alguns inquéritos feitos no Brasil podem dar alguma ideia da frequência das infecções humana e canina. A percentagem de cães infectados tem sido quase sempre mais alta ou muito mais alta que a de pessoas doentes, mas, como a população humana é muito maior que a canina, os percentuais não exprimem o número global de fontes de infecção humanas ou caninas, podendo o número de pessoas parasitadas ser maior que o de cães. A Comissão Chagas, examinando pràticamente a totalidade da população humana e canina de um foco de calazar no estado do Pará, encontrou 8 pessoas e 7 cães infectados (34). Dezasseis meses de observação numa área endémica no Noroestte do estado do Ceará revelaram 52 infecções humanas e 7 caninas (43, 59); em outra região do mesmo estado do Ceará (o vale do rio Jaguaribe) foram registrados durante dez meses 56 casos humanos e 11 caninos (9); no estado do Piauí, em área de onde provieram 95 doentes, foram encontrados 74 cães infectados (4); em algumas oportunidades não se conseguiu descobrir cães infectados em sítios onde adoeceram pessoas (31). Convém mencionar que algumas dessas observações foram feitas no decorrer de surto epidémico de calazar entre a população humana, não se podendo desprezar a possibilidade de ter ocorrido anteriormente um surto semelhante entre a população canina; de qualquer modo, isso sugere que, em certas circunstâncias, o homem pode ser numèricamente reservatório mais importante que o cão (49). Por outro lado, estudos feitos nos arredores da cidade litorânea de Fortaleza, estado do Ceará, permitiram encontrar 2 casos humanos contra 39 caninos, com diagnóstico parasitológico (6), condição semelhante à que ocorre em alguns focos do Mediterrâneo. Também nos focos do estado de Minas Gerais a incidência do calazar canino é considerada significantemente mais alta do que a humana: assim, nos municípios de Turumirim e Café, onde

foram registrados 15 casos humanos, a pesquisa de parasitas em cães revelou 21 infecções; a RFC foi negativa em 386 pessoas examinadas após a retirada dos doentes citados e positiva em 68 cães (20).

Quanto à raposa *Lycalopex vetulus,* a frequência de sua infecção nas zonas endémicas é difícil de avaliar. Esse animal habita a maior parte mas não toda a área de incidência de calazar no Brasil e não se encontra em outras áreas endémicas do continente. Na zona rural do Nordeste brasileiro essa raposa é um mamífero muito frequente, mas não temos meios de calcular quantitativamente a sua população. No Noroeste do estado do Ceará, de 10 exemplares capturados em focos endemo-epidémicos de calazar, 3 estavam infectados, e de 23 apanhados em zonas de baixa endemicidade, 1 mostrou-se parasitado (43, 49). Não podemos, entretanto, comparar a percentagem de infecção encontrada entre as raposas com a verificada entre pessoas ou cães, não sòmente porque o número de raposas que se conseguiu capturar e examinar foi relativamente pequeno, como também porque as raposas infectadas, doentes e portanto menos ágeis, talvez se deixem apanhar com mais facilidade que os animais sadios.

Individualmente, uma raposa parasitada é fonte de infecção tão importante quanto um cão. Tanto quanto nele, sua infecção cutânea é frequente e intensa — 3 dentre 4 exemplares parasitados apresentavam leishmânias na pele, em 2 o parasitismo era mais intenso do que em qualquer dos cães examinados e a única raposa experimentada infectou todos os 10 *P. longipalpis* que a picaram, neles obtendo-se infecção rica, inclusive com bloqueio mais intenso da faringe do que o observado em flebótomos alimentados em pessoas ou mesmo em cães infectados (43, 49, 58).

Resumindo os actuais conhecimentos sobre os reservatórios do calazar americano, julgamos que o homem e o cão são suficientes para manter o ciclo doméstico nas áreas endemo-epidémicas, sendo aí o cão o principal reservatório, embora o homem possa desempenhar um apreciável papel, principalmente durante as epidemias. Quanto à raposa, não é essencial na transmissão do calazar nos focos endemo-epidémicos, mas em algumas dessas áreas pode ser encarada como um reservatório importante. Òbviamente, nas cidades ela não entra na cadeia de transmissão. Mas é, por sua vez, provàvelmente responsável por casos esporádicos de calazar que têm sido assinalados em várias áreas, sem relação aparente com outros casos, humanos ou

caninos (24, 59). Seus hábitos migratórios possìvelmente lhe permitirão também introduzir a moléstia em zonas antes livres, mas com população humana e canina e de transmissores em proporções adequadas para a criação e a manutenção de novos focos (59). A raposa não parece ser entretanto o reservatório ancestral e primitivo do agente do calazar americano, procurado sem sucesso pela Comissão Chagas. A pronunciada acção patogénica da *L. donovani* para a raposa — que pode adoecer gravemente e morrer da infecção — não fala a favor de um parasitismo secular, o que teria provàvelmente resultado num relativo equilíbrio entre o parasita e o hospedeiro. Na raposa a infecção, aparentemente, evolui do mesmo modo que no cão e os sintomas apresentados são da mesma ordem. Uma das quatro encontradas infectadas tinha aspecto sadio; duas outras estavam emagrecidas, com vivacidade e agressividade bem diminuídas, sendo que uma exibia pequena úlcera no lábio e a outra apresentava queda completa dos pêlos da face dorsal do focinho; a última das quatro tinha sintomas idênticos aos dos cães nas fases avançadas da doença: emagrecimento pronunciado, apatia, paresia das patas posteriores, pêlo eriçado, depilação difusa discreta; o focinho inteiramente glabro, edemaciado, lustroso; edema das patas, queratite bilateral e diarreia (43, 49). Sendo assim, não se pode excluir a hipótese de ter sido o calazar americano importado do Velho Mundo por intermédio de cães ou de indivíduos humanos parasitados, tendo secundàriamente atingido um canídio campestre muito susceptível e que frequentemente se aproxima das casas, como a raposa.

Outros reservatórios da *L. donovani* no continente americano têm sido procurados. No Brasil, a Comissão Chagas encontrou 1 gato doméstico infectado, num foco do estado do Pará (34). Não se suspeita, entretanto, que esse animal seja reservatório habitual, pois é raramente sugado pelo transmissor (43) e sua infecção experimental é difícil; recentemente não conseguimos infectar 3 gatos recém-nascidos pela inoculação intraperitoneal de emulsão de vísceras de cão parasitado, rica em leishmânias e que infectou *hamsters* muito fàcilmente. O exame, por punção de fígado e biopsia ou necroposia, de 142 gatos de uma área endémica do Noroeste do Ceará foi negativo (49).

Reservatórios campestres têm sido pesquisados, no Brasil, em diferentes áreas endémicas e em várias ocasiões, mas, fora a raposa,

nenhum outro animal demonstrou albergar leishmânias atribuíveis à *L. donovani.*

A Comissão Chagas examinou num foco do estado do Pará numerosos mamíferos silvestres; nessa ocasião em 2 «ratos sauiás» *(Proechimys guyanensis oris)* foram encontradas em esfregaços de baço formações suspeitas de leishmânias, mas não se conseguiu isolar parasitas pela semeadura do sangue e vísceras de outros exemplares da mesma espécie em meios de cultura nem se obteve infecção experimental de um exemplar inoculado com culturas de doentes humanos (34). Nas vísceras de «preguiças-reais» *(Choloepus didactylys)* da mesma região foram achadas leishmânias morfològicamente semelhantes às humanas (42); entretanto, como esse mamífero alberga também um tripanossoma e uma critídia endoeritrocítica assinalada com o nome de *Endotrypanum schandinni* (84), é possível que as formas em leishmânia constituíssem a fase de multiplicação daqueles tripanossomídeos, cujo ciclo evolutivo ainda é desconhecido (42). No Nordeste brasileiro foram ainda examinados muitos mamíferos campestres, com resultado negativo (43, 49, 100). Não se pode, entretanto, excluir a possibilidade da existência de outros hospedeiros da *L. donovani* no continente americano, inclusive de reservatórios silvestres primitivos. A pesquisa destes tem sido feita pelo exame de esfregaços ou cortes de pele, fígado, baço, medula óssea ou outras vísceras e certamente não tem sido efectuada em escala suficientemente grande. Nesse particular convém lembrar que nas pesquisas que estão sendo recentemente realizadas no Panamá, para a descoberta de reservatório silvestre da leishmaniose tegumentar, é feita a semeadura de sangue dos animais em meios de cultura, o que já resultou no encontro de exemplares de «ratos-de-espinho» *(Proechimys semispinosus panamensis)* portadores de parasitos que dão leishmânias e leptomonas indistinguíveis morfològicamente da *L. brasiliensis* e que, por inoculação, produziram úlcera leishmaniótica num voluntário humano (72). Além disso, no Quénia, em área endémica de calazar, foi possível infectar *hamster* pela inoculação de emulsão do baço de um esquilo *(Xerus rutilus)*, espécie de animal da qual muitos exemplares haviam anteriormente sido negativos pelo exame directo (71).

Transmissores. — No continente americano, estudos referentes aos transmissores do calazar têm sido feitos principalmente no Brasil.

A Comissão Chagas, embora não tenha incriminado directamente o *Phlebotomus longipalpis* como vector, mencionou a sua presença na maioria dos focos estudados (27, 28, 30, 34, 40, 44, 79, 100). A infecção experimental do *P. longipalpis* em cães fora também assinalada por membros daquela Comissão (25, 26, 63), que entretanto também infectaram experimentalmente o *P. intermedius* e, com a inoculação de triturado deste, conseguiram a transmissão experimental a animais de laboratório (94), reconhecendo, embora, que o papel mais importante deveria caber à primeira espécie (25).

Estudos recentes permitem incriminar definitivamente o *P. longipalpis* como transmissor habitual do calazar nas áreas endémicas conhecidas do Brasil e o único vector de importância epidemiológica em quase todas elas. Os seguintes factos relacionados com o *P. longipalpis* conduzem a essa conclusão:

*a*) Sua distribuição geográfica nesse país coincide com a do calazar, tanto qualitativa como quantitativamente, mesmo quando se comparam zonas muito próximas, porém com graus de endemicidade bem diversos (43, 50, 59);

*b*) Em quase todos os focos de calazar o *P. longipalpis* é, de longe, a espécie predominante de flebótomo. No Noroeste do estado do Ceará constituiu 97 % de mais de 134 000 flebótomos apanhados (43, 50, 59); no vale do rio Jaguaribe, 92 % (6), e em Fortaleza, no mesmo estado, 95,7 % (6, 9); no estado de Piauí, 99,9 % (4, 43); no principal foco do estado da Baía, Jacobina, 99 % (77). Em muitas ocasiões foi o único díptero hematófago encontrado em habitações humanas de áreas endémicas;

*c*) Sua densidade nos focos de calazar é geralmente muito grande e, embora não seja estritamente doméstico, pode ser capturado em números elevados — às centenas ou aos milhares — no interior das casas (43);

*d*) Alimenta-se em diversos animais de sangue quente, sugando àvidamente o homem e o cão e picando também a raposa (43);

*e*) Sua infecção experimental foi fàcilmente conseguida em doentes humanos (49, 57), em cães infectados (25, 26, 49, 56, 63) e numa raposa parasitada (49), tendo o protozoário nele evoluído em qualquer dos casos, de modo idêntico, produzindo, inclusive, o bloqueio da faringe;

*f*) Foi encontrado naturalmente infectado, por leptomonas idênticas às das leishmânias humanas e de localização anterior no tubo digestivo, em dois focos brasileiros de calazar: no Noroeste do Ceará (43, 59) e em Jacobina, estado da Baía (76, 77);

*g*) As demais espécies de flebótomos assinaladas em quase todas as áreas endémicas de leishmaniose visceral no Brasil ou são raras, ou não frequentam as casas, ou não costumam alimentar-se em reservatórios conhecidos da *L. donovani*. É possível que uma ou outra dentre elas sirva ocasionalmente de vectora, mas não cremos que se lhes possa imputar responsabilidade pela manutenção da endemia.

Em focos de calazar do Centro-Leste do Brasil, no estado de Minas Gerais, embora o *P. longipalpis* seja a espécie mais numerosa, outro flebótomo, o *P. cortellezzii*, é também abundante, inclusive nas proximidades das casas, sendo necessários mais estudos para esclarecer seu papel na epidemiologia da moléstia aí (81).

Nos outros países do continente onde o calazar incide ainda são escassos os estudos sobre seus transmissores. O *P. longipalpis* já foi assinalado no México, Guatemala, Venezuela, Colômbia, Paraguai e Argentina (13, 61, 64), mas não tem sido encontrado por ocasião dos poucos inquéritos epidemiológicos que têm sido feitos nas localidades onde ocorreram os casos esporádicos de calazar registrados nesses países, excepto na Guatemala, onde, numa inspecção recentemente realizada em sítios do departamento El Progreso, onde ocorreram os dois únicos casos humanos de calazar registrados nesse país, os flebótomos capturados foram identificados, em sua quase totalidade, como *P. longipalpis* (64). Em dois focos do estado de Carabobo, Venezuela, os muitos flebótomos apanhados foram determinados como *P. panamensis* (99). Nesse mesmo país, em área endémica de leishmaniose tegumentar do vale do Yaracuy, foi encontrado o *P. longipalpis* naturalmente infectado por leptomonas então atribuídas à *L. brasiliensis* (98).

Desejamos chamar a atenção para um aspecto da transmissão do calazar que merece mais investigações: a possibilidade de transmissão directa entre animais, sem a intervenção de flebótomos. No Brasil há localidades com densidade muito pequena de flebótomos e raros casos humanos, porém muitos caninos, de leishmaniose visceral. Como esses flebótomos não demonstram nítida preferência para se alimentar em cães, é-se levado a pensar na transmissão directa entre esses ani-

mais (6, 20, 43, 48, 91). A possibilidade da transmissão pelo coito não nos parece ter sido convenientemente investigada, convindo mencionar que num dos cães naturalmente infectados que examinámos encontrámos inflamação e pequenas ulcerações na glande e prepúcio, com acentuado parasitismo por leishmânias (43). Posteriormente, o exame anátomo-patológico do pénis de um cão com leishmaniose visceral de um foco do estado de Minas Gerais revelou abundantes histiócitos contendo leishmânias e localizados principalmente na região periuretral e na camada subepitelial da glande, o que levou o autor do achado a pensar numa possível relação entre esse facto e o problema da transmissão directa da leishmaniose visceral canina (20).

População susceptível. — No continente americano casos de leishmaniose visceral têm sido encontrados com idade variando desde 45 dias até mais de 60 anos; porém, conforme verificado desde os estudos da Comissão Chagas no Brasil, o calazar neotropical ocupa, neste particular, um lugar intermediário aos tipos «indiano» e «mediterrâneo» (32, 33). Num grupo de 231 doentes recentemente observados no estado do Ceará (2) a incidência do calazar por grupo etário foi a seguinte:

Quadro 3

| Grupo etário, em anos | Percentagem de casos |
|---|---|
| Menos de 1 | 8,7 |
| 1 a 4 | 58,3 |
| 5 a 9 | 17,4 |
| 10 a 14 | 6,3 |
| 15 a 19 | 3,7 |
| 20 a 29 | 3,1 |
| 30 ou mais | 2,5 |

Por aí se verifica que perto de 85 % dos doentes têm menos de 10 anos de idade. O primeiro quinquénio concorreu com 67 % dos casos, em vez dos 93 % atribuídos ao tipo mediterrâneo (87), e o grupo de 5 a 14 anos, que na Índia atinge cerca de 60 % dos pacientes (87), na amostra acima compreendeu menos de 24 %. Resultados

próximos destes têm sido verificados em grupos menores de doentes brasileiros (9, 108). Nas áreas de baixa endemicidade a diferença de incidência entre adultos e crianças é menor, o mesmo acontecendo em áreas que parecem ter sido invadidas recentemente, como julgamos serem alguns dos focos há pouco estudados no estado de Minas Gerais (81).

Têm sido observados mais pacientes do sexo masculino que do feminino: em 176 doentes do Ceará a proporção foi de 60 % para 40 % (2); em outras casuísticas os pacientes masculinos têm constituído de 53 % até 100 % do total (8, 9, 81, 108). Não sabemos se essa predominância é real e não conhecemos estudos visando avaliar possíveis influências de raça, nível económico, hábitos ou outros factores individuais na prevalência da leishmaniose visceral neotropical.

O MEIO AMBIENTE. — Pouco se sabe ainda sobre a influência das condições ambientes na disseminação do calazar americano. Tais condições agem indirectamente, influindo sobre a densidade dos transmissores, a distribuição das populações susceptíveis e as oportunidades de acesso daqueles a estas.

Os casos de calazar americano têm sido assinalados em áreas de climas equatorial, tropical ou semiárido, raramente em clima subtropical, como no Chaco argentino, onde a temperatura no Inverno vai abaixo de 0°C (106). Quase todos os focos se situam em zonas com humidade média acima de 70 %, mas algumas das principais áreas endémicas ficam em trechos com pluviosidade inferior a 1000 mm anuais. Têm sido achados casos esporádicos em altitudes superiores a 800 m, porém a grande maioria incide abaixo de 500 m.

A leishmaniose visceral americana foi considerada a princípio exclusivamente rural ou silvestre (32, 33, 41). Hoje sabe-se que ela é predominantemente rural, mas também pode ser contraída em zonas suburbanas e urbanas. Têm sido encontrados casos autóctones em cidades brasileiras, como Sobral (48, 96), Russas e Jaguaruana (9), no estado do Ceará, e Jacobina, no estado da Baía (97). Nessas mesmas cidades, bem como em Fortaleza, estado do Ceará, foram encontrados cães infectados (6, 9, 48, 75, 77). Numa área endémica investigada no Nordeste brasileiro (Sobral, Ceará) foi verificado que 96 % dos doentes contraíram a moléstia em zona rural e 4 % em cidades (43, 59). Há casos particulares em que a situação e a topografia

de pequenas cidades do interior, propiciando maior proliferação dos vectores e seu contacto com população mais densa, as tornam focos mais importantes do que a zona rural vizinha, conforme observado em Jacobina (43).

A influência do tipo de região na prevalência do calazar americano foi assinalada desde os trabalhos pioneiros da Comissão Chagas, no Brasil: no Nordeste e Leste a doença ocorria nas zonas do litoral e da mata e nas serras interioranas com vegetação florestal, isto é, nos trechos menos secos da região; na Amazónia, florestada e húmida, ela incidia apenas em áreas de «terra firme», isto é, de terrenos não alagáveis, e não nas de «várzea» que margeiam os grandes rios, onde reside a maior parte da população rural, mas onde a inundação diária do solo pela água das marés impede o desenvolvimento das larvas dos flebótomos (29, 30, 32, 34).

Mais recentemente, pesquisas efectuadas no Noroeste do estado do Ceará mostraram que nessa área de planícies semiáridas entremeadas de serras os principais focos de calazar se localizam nos estreitos vales entre as serras ou nos sopés destas, mais húmidos, mais ricos em vegetação e menos ventilados que as planícies e menos frios que o alto das serras (43, 53, 59). O mesmo foi verificado em um foco do Norte do estado de Piauí (43) e na zona de Jacobina, estado da Baía (97). Também no estado de Minas Gerais os focos de calazar se situam na confluência do declive de serras (81).

Outra zona preferencial da endemia no Nordeste brasileiro é representada pelas baixadas marginais dos rios (43).

Nos vales e sopés de serras o calazar não só é mais frequente, como também pode assumir aspecto endemo-epidémico. Os casos lá observados, geralmente, podem ser relacionados com outros, humanos ou caninos, ocorrendo no mesmo domicílio ou em sua vizinhança. Não é raro adoecerem duas, três ou mais pessoas na mesma casa, e em algumas de tais áreas parecem ter ocorrido epidemias que dizimaram quase toda a população de menos de 5 anos de idade (43, 59).

A influência estacional está pouco estudada na Região Neotropical, porém algumas observações a respeito foram feitas no estado do Ceará, Brasil. Aí ocorrem apenas duas estações: uma chuvosa, que inclui mais de 90 % da precipitação anual e se estende de Janeiro a Junho, mais húmida, menos ventilada e de temperatura um pouco mais baixa, e outra seca, ocupando o resto do ano. Uma investigação

sobre o mês em que se iniciaram os sintomas em 1300 pacientes revelou que a maioria destes adoeceu no início da estação seca, com o pico de incidência em Agosto (3), cerca de três meses após o cume da densidade do *Phlebotomus longipalpis*, que é em Maio e que, por sua vez, ocorre cerca de dois meses depois do acme da pluviometria, em Março (50). O primeiro desses intervalos corresponde provàvelmente ao período médio de incubação da doença. Durante a estação chuvosa a proliferação e a sobrevivência dos flebótomos são favorecidas não só pelo aumento da humidade, como também pelo crescimento da vegetação, que lhes propicia mais abrigo e melhores criadouros. Em certas áreas do Nordeste brasileiro outro facto soma-se à maior produção de flebótomos para explicar a maior intensidade de transmissão durante a estação chuvosa. Com a chegada das chuvas, nos vales e sopés das serras, que são, como vimos, os focos de maior endemicidade do calazar, aumenta extraordinàriamente a densidade de moscas hematófagas e tabânidas, que, durante o dia e ao ar livre, agridem vorazmente os animais domésticos; isso obriga, nessa época, os moradores a transferir o seu gado para a planície; esse facto restringe as fontes de hematofagia para os flebótomos justamente numa ocasião em que estes são mais abundantes, daí resultando um ataque mais frequente ao homem (50).

Já referimos as ondas epidémicas que ocorrem em áreas de maior endemicidade do calazar americano, conforme observado no Nordeste do Brasil. É possível que tais surtos tenham relação com o fenómeno das secas periódicas que atingem essa região, trazendo como consequência uma diminuição na resistência da população.

## PROFILAXIA

Sòmente no Brasil, onde em certas áreas a leishmaniose visceral é considerada importante problema sanitário, estão sendo efectuadas medidas de profilaxia visando especìficamente essa moléstia. Tais medidas destinam-se a combater os transmissores e a reduzir as fontes de infecção.

As primeiras consistem na aspersão domiciliária das áreas endémicas com DDT em emulsão ou suspensão aquosa a 3 %, na razão de 2 g por metro quadrado de superfície, uma vez por ano. A asper-

são é feita não só nas casas, mas também nos abrigos de animais domésticos (alojamentos fechados, currais, galinheiros), pois o *Phlebotomus longipalpis* invade estes mais abundantemente ainda do que os domicílios, sendo aliás encontrado em proporção bem menor nos abrigos extradomésticos, tais como ocos de árvores, locas de pedras, etc. (43, 51, 52). O efeito desse rociamento sobre a densidade do *P. longipalpis* pode ser avaliado através de experiência realizada no estado do Ceará (54): de quatro áreas endémicas rurais comparáveis e onde foram prèviamente obtidos índices de densidade de flebótomos, duas tiveram suas casas e abrigos de animais domésticos dedetizados, servindo as outras duas como testemunhas. A determinação mensal posterior de índices de densidade do vector mostrou que, logo após a dedetização, os flebótomos pràticamente desapareceram das casas tratadas e ao fim de quatro meses a sua densidade nelas mantinha-se muito baixa; nos abrigos de animais domésticos a densidade também caiu muito, mas bem menos que dentro das casas, e pelo terceiro mês já não havia mais aí evidência da influência da dedetização; entretanto, as capturas feitas ao ar livre, em isca animal, revelaram que a densidade de flebótomos apanhados nestas condições não foi infuenciada pela dedetização.

Esta experiência leva a crer que a transmissão intradomiciliária do calazar poderá ser muito reduzida pela dedetização conforme praticada. Tal modalidade de transmissão deve ser a mais frequente, visto que o *P. longipalpis* suga mais durante o período nocturno, quando a população humana está recolhida às casas, principalmente aquela parte da população que é a mais susceptível, isto é, o grupo etário de menos de 5 anos. Por outro lado, ignora-se a importância da transmissão extradomiciliária do calazar. Que ela ocorre, pode ser comprovado pelo facto de que um dos exemplares de *P. longipalpis* encontrados naturalmente infectados no Ceará fora capturado sugando ao ar livre (59). Mas, em vista do que se tem observado com a malária, que vem desaparecendo de áreas cujas casas são dedetizadas mesmo quando os transmissores são anofelinos pouco domésticos, e mesmo a julgar pelas observações feitas num foco de leishmaniose tegumentar no Brasil (70), é de crer que, no calazar, a própria transmissão extradomiciliária finalmente se esgote, à custa da mera dedetização das casas e abrigos de animais domésticos.

Medidas antilarvárias não têm sido usadas na profilaxia do calazar no Brasil, visto que, embora algumas larvas e pupas de *P. longipalpis* já tenham sido encontradas em natureza (43, 52), não são ainda suficientemente conhecidos os seus criadouros.

Para reduzir as fontes de infecção, procura-se tratar sistemàticamente os casos humanos e eliminar os cães doentes, desde que, conforme vimos, ambos esses hospedeiros funcionam como reservatórios importantes do agente causal. Para o diagnóstico dos casos, os médicos das áreas endémicas têm sido alertados quanto aos sintomas do calazar, cuja tratamento — feito com antimoniato-N-metil-glucamina ou com gluconato de sódio e antimónio — é obtido grátis em pequenos hospitais ou postos especializados. Para a supressão dos cães doentes o critério tem sido examinar sistemàticamente os cachorros dos focos e sacrificar todos aqueles cuja infecção foi comprovada parasitològicamente pelo exame de esfregaços de pele ou de fígado obtido por punção, bem como aqueles nos quais não se encontrou leishmânias, porém cuja sintomatologia foi muito suspeita. Com esse critério escapa uma porporção de cães infectados, de parasitismo escasso e não revelável a um único exame, os quais seriam entretanto menos importantes do ponto de vista epidemiológico.

Para estudar a possibilidade de combater o reservatório campestre conhecido são necessárias investigações visando melhor conhecer os hábitos da raposa *Lycalopex vetulus*.

## BIBLIOGRAFIA


1. Albuquerque, A. F. R., Brito, S., e Morais, M. O. — «Importante foco de mal de Chagas e de leishmaniose visceral americana no vale do Cariri (estados do Ceará e Pernambuco)», *O Hospital,* **21**: 61-69, 1942.
2. Alencar, J. E. — «Leishmaniose visceral no Novo Mundo», *Publicações Médicas,* **196**: 71-87, 1956.
3. Alencar, J. E. — «Calazar no Nordeste Septentrional Brasileiro», *Ceará Méd.* (em publicação), 1958.
4. Alencar, J. E. — «Incidência da leishmaniose canina no Piauí. Meios diagnósticos utilizados num inquérito», *Ceará Méd.* (em publicação), 1958*a*.
5. Alencar, J. E., e Aragão, T. C. — «Leishmaniose visceral no Ceará. Sintomas observados em 174 casos. Diagnóstico clínico», *Publicações Médicas,* **28**: 7-20, 1957.

6. Alencar, J. E., Cantidio, W. M., e Cavalcante, D. N. — *Calazar em Fortaleza.* Apres. XIII Congr. Brasil. Hig., Fortaleza, Ceará, 1956.
7. Alencar, J. E., e Coelho Neto, B. — *Leishmaniose canina no Ceará,* Apres. XIII Congr. Brasil. Hig., Fortaleza, Ceará, 1956.
8. Alencar, J. E., Dinis, J. O., Pessoa F.º, F. P., Aragão, T. C., e Correia, T. A. — *Alguns focos de calazar no Piauí.* Apres. XIII Congr. Brasil. Hig., Fortaleza, Ceará, 1956.
9. Alencar, J. R., Holanda, D., e Cavalcante, J. D. N. — «Calazar no vale do Jaguaribe, Ceará, 1955», *Rev. Brasil. Malariol. & D. Trop.,* **8**: 33-47, 1956.
10. Almeida, E. — «Um caso de leishmaniose visceral na Paraíba», *Brasil Méd.,* **59**: 82-83, 1945.
11. Aragão, T. C. — *Surto de leishmaniose visceral na zona norte do estado do Ceará.* Comun. feita à 2.ª Reunião de Saúde Pública do Estado do Ceará, 1953.
12. Arruda, V., Costa, S. C., Nahas, S., e Rosenfeld, G. — «Leishmaniose visceral americana. Constatação de dois casos», *Brasil Méd.* **63**: 64-65, 1949.
13. Barretto, M. P. — «Nova contribuição para o estudo da distribuição geográfica dos flebótomos americanos *(Diptera, Psychodidae)*», *Arq. Hig. Saúde Públ. S. Paulo,* **13**: 211-226, 1950.
14. Barros, O. M., e Maciel, P. — «Leishmaniose visceral americana (2 casos de um novo foco na Baía)», *Rev. Clín. S. Paulo,* **27**: 55-66, 1951.
15. Barros, O. M., e Rosenfeld, G. — «Leishmaniose visceral americana. Um caso da Bolívia», *Rev. Clín. S. Paulo,* **11**: 91-99, 1942.
16. Barros, O. M., e Rosenfeld, G. — «Leishmaniose visceral americana. Novo caso», *Rev. Clín. S. Paulo,* **15**: 97-102, 1944.
17. Bloch, M., y Guillen, G. — «Kala-azar en El Salvador», *Arch. Col. Med. El Salvador,* **3**. 90-96, 1950.
18. Boggino, J., y Maas, L. C. — «Primer caso ciertamente autóctono de leishmaniasis visceral», *Ann. Fac. Cienc. Méd. Univ. Nac. Asunción,* **5**: 319-324, 1945.
19. Bogliolo, L. — «Nova contribuição ao conhecimento da anatomia patológica da leishmaniose visceral. A propósito de um caso brasileiro e com especial referência à fibrose hepática leishmaniótica», *O Hospital,* **50**: 394-440, 1956.
20. Brener, Z. — *Calazar canino em Minas Gerais.* Tese de doutoramento, Belo Horizonte, mimeografada, 90 pp., 1957.
21. Cabrera, M. A., y León, J. R. — «Historia del primer caso clínico de leishmaniasis visceral descubierto en Guatemala», *Public. Inst. Investig. Cientif.,* **2**, Guatemala, 36 pp., 1949.
22. Caldeira, O. V., Bogliolo, L. — «Leishmânides dérmicos na leishmaniose visceral (calazar) no Brasil», *O Hospital,* **51**: 193-205, 1957.
23. Cançado, J. R., Gama, G., Mourão, O. G., Brener, Z., Antonaccio, F., Lana, G., e Romeiro V. — «Calazar autóctone em Minas Gerais», *O Hospital,* **50**: 373-392. 1956.
24. Carvalho, S. B., e Pessoa, S. B. — «Dois novos casos de leishmaniose visceral na Paraíba», *An. Fac. Med. Paraíba,* **1**: 91-105, 1956.

25. Chagas, A. W. — «Infecção do *Phlebotomus intermedius* pela *Leishmania chagasi*», *Brasil Méd.*, **53**: 1-2, 1939.
26. Chagas, A. W. — «Criação de flebótomos e transmissão experimental da leishmaniose visceral americana», *Mem. Inst. Osw. Cruz*, **35**: 328, 1940.
27. Chagas, E. — «Primeira verificação em indivíduo vivo da leishmaniose visceral no Brasil», *Brasil Méd.*, **50**: 221-222, 1936.
28. Chagas, E. — «Estudos sobre as grandes endemias do Brasil», *O Hospital*, **14**: 1323-1353, 1938.
29. Chagas, E., e Castro, G. O. — «Observações sobre o clima, a flora e a fauna dos focos de leishmaniose», *Mem. Inst. Osw. Cruz*, **32**: 358-370, 1937.
30. Chagas, E., Castro, G. O., e Ferreira, L. C. — «Considerações sobre a leishmaniose visceral americana», *Mem. Inst. Osw. Cruz*, **32**: 381-389, 1937.
31. Chagas, E., e Chagas, A. W. — «Notas sobre a epidemiologia da leishmaniose visceral em Mato Grosso», *O Hospital*, **13**: 471-480, 1938.
32. Chagas, E., Cunha, A. M., Castro, O., Ferreira, C., e Romaña, C. — «Leishmaniose visceral americana. Relatório dos trabalhos realizados pela comissão encarregada dos estudos sobre a leishmaniose visceral americana em 1936», *Mem. Inst. Osw. Cruz*, **32**: 321-385, 1937.
33. Chagas, E., Cunha, A. M., Ferreira, L. C., Deane, L., Deane, G., Guimarães, F. N., Paumgartten, M. J., e Sá, B. — «Leishmaniose visceral americana. Relatório dos trabalhos realizados pela comissão encarregada dos estudos da leishmaniose visceral americana em 1937», *Mem. Inst. Osw. Cruz*, **33**: 89-229, 1938.
34. Chagas, E., Ferreira, L. C., Deane, G., Deane, L., e Guimarães, F. N. — «Leishmaniose visceral americana. II. Estudos epidemiológicos», *Mem. Inst. Osw. Cruz*, **33**: 138-206, 1938.
35. Chagas, E., e Guimarães, F. N. — «Leishmaniose visceral americana. III. Processos patogénicos», *Mem. Inst. Osw. Cruz*, **33**: 207-229, 1938.
36. Coutinho, A., e Lira, A. — «Leishmaniose visceral em Pernambuco. Considerações clínicas e hematológicas sobre um novo caso», *Rev. Brasil. Med.*, **11**: 89-97, 1954.
37. Cunha, A. M. — «Infections expérimentales obtenues en partant de la leishmaniose viscérale américaine», *C. R. Soc. Biol.*, **129**: 428-430, 1938.
38. Cunha, A. M. — «Infecções experimentais na leishmaniose visceral americana», *Mem. Inst. Osw. Cruz*, **33**: 581-598; 1938.
39. Cunha, A. M. — «Infecção da pele na leishmaniose visceral experimental do cão», *Brasil Méd.*, **52**: 1071-1072, 1938.
40. Cunha, A. M., e Chagas, E. — «Études sur la leishmaniose viscérale du Brésil. Verification du parasite par la ponction de la rate et du foie; culture et aspect morphologique; considérations d'ordre générale», *C. R. Soc. Biol.*, **123**: 709-711, 1936.
41. Deane, L. — «Leishmaniose visceral neotrópica», *O Hospital*, **13**: 315-321, 1938.

42. Deane, L. M. — «Comentário ao trabalho de C. A. Hoare, *The relationship of the haemoflagellates*», *Proc. 4th Internat. Congr. Trop. Med. & Malaria*, **2**: 1117, Washington, 1948.
43. Deane, L. M. — *Leishmaniose visceral no Brasil. Estudos sobre reservatórios e transmissores realizados no estado do Ceará*. Tese de docência-livre apres. à Fac. Med. Univ. S. Paulo, Brasil, S. N. E. S., Rio de Janeiro, 162 pp., 1956,
44. Deane, L., e Deane, G. — «Estudos sobre a leishmaniose visceral americana. I. Aspectos clínicos da doença», *O Hospital*, **17**: 189-199, 1937.
45. Deane, L., Deane, G., Paumgartten, M. J., e Sá, B. — «Leishmaniose visceral americana. I. Estudos clínicos. Terapêutica», *Mem. Inst. Osw. Cruz*, **33**: 95-137, 1938.
46. Deane, L. M., e Deane, M. P. — «Encontro de leishmânias nas vísceras e na pele de uma raposa em zona endémica de calazar nos arredores de Sobral, Ceará», *O Hospital*, **45**: 419-421, 1954.
47. Deane, L. M., e Deane, M. P. — «Encontro de cães naturalmente infectados por *Leishmania donovani* no Ceará», *O Hospital*, **45**: 703-707, 1954*a*.
48. Deane, L. M., e Deane, M. P. — «Leishmaniose visceral urbana (no cão e no homem) em Sobral, Ceará», *O Hospital*, **47**: 75-87, 1955.
49. Deane, L. M., e Deane, M. P. — «Observações preliminares sobre a importância comparativa do homem, do cão e da raposa *(Lycalopex vetulus)* como reservatórios da *Leishmania donovani* em área endémica de calazar no Ceará», *O Hospital*, **48**: 61-76, 1955*a*.
50. Deane, L. M., e Deane, M. P. — «Sobre a biologia do *Phlebotomus longipalpis* transmissor da leishmaniose visceral em uma zona endémica do estado do Ceará. I. Distribuição, predominância e variação estacional», *Rev. Brasil. Biol.*, **15**: 83-95, 1955*b*.
51. Deane, L. M., e Deane, M. P. — «Sobre a biologia do *Phlebotomus longipalpis*, transmissor da leishmaniose visceral em uma zona endémica do estado do Ceará. II. Frequência domiciliária e hábitos alimentares», *Rev. Paulista Med.*, **47**: 457, 1955*c*.
52. Deane, L. M., e Deane, M. P. — «Observações sobre abrigos e criadouros de flebótomos no Noroeste do estado do Ceará», *Rev. Brasil. Malariol. & D. Trop.*, **9**: 225-246, 1957.
53. Deane, L. M., Deane, M. P., e Alencar, J. E. — «Tipo de região e prevalência da leishmaniose visceral em uma área endémica do Ceará», *Rev. Paulista Med.*, **46**: 130-131, 1955.
54. Deane, L. M., Deane, M. P., e Alencar, J. E. — «Observações sobre o combate ao *Phlebotomus longipalpis* pela dedetização domiciliária em focos endémicos de calazar no Ceará», *Rev. Brasil. Malariol. & D. Trop.*, **7**: 131-141, 1955.
55. Deane, M. P. — 1939 — *In* Alencar, 1956.
56. Deane, M. P., e Deane, L. M. — «Infecção natural do *Phlebotomus longipalpis* por leptomonas, provàvelmente de *Leishmania donovani*, em um foco de calazar no Ceará, *O Hospital*, **45**: 697-702, 1954.

57. Deane, M. P., e Deane, L. M. — «Infecção experimental do *Phlebotomus longipalpis* em caso humano de leishmaniose visceral», *O Hospital,* **46**: 487-489, 1954*a*.

58. Deane, M. P., e Deane, L. M. — «Infecção experimental do *Phlebotomus longipalpis* em raposa *(Lycalopex vetulus)* naturalmente parasitada pela *Leishmania donovani*», *O Hospital,* **46**: 651-653, 1954*b*.

59. Deane, M. P., e Deane, L. M. — «Observações sobre a transmissão da leishmaniose visceral no Ceará», *O Hospital,* **48**: 347-364, 1955.

60. Deane, M. P., e Deane, L. M. — «Sobre o encontro de leishmanias na pele de doentes de calazar», *Rev. Paulista Med.,* **50**: 204, 1957.

61. Duret, J. B. — «Notas sobre flebótomos argentinos», *Rev. Sanit. Milit. Argent.,* **51**: 534-536, 1952.

62. Fernandez Ithurrat, E. — «Un caso de leishmaniose visceral autóctona», *El Dia Médico,* **13**: 14, 1941.

63. Ferreira, L. C., Deane, L., e Mangabeira, F.°, O. — «Infecção de *Phlebotomus longipalpis* pela *Leishmania chagasi*», *O Hospital,* **14**: 2-3, 1938.

64. Figueroa García, L. N. — «Leishmaniosis visceral (kala-azar). Segundo caso clínico descubierto en Guatemala», *Publ. Instit. Investig. Cientif.,* n.° 11, Guatemala, 1948.

65. Fonso, C., y Ink, J. — «Sobre un caso autóctono de leishmaniosis visceral. Estudio clínico-experimental», *Rev. Medic. y Cienc. Af.,* **9**: 36-43, 1947.

66. Franco Palacios, M. — «Kala-azar infantil en el Estado de Carabobo», *Rev. Policl. Caracas,* **14**: 526-531, 1945.

67. Gast G., A., y Rengifo, S. — «Leishmaniasis visceral. Estudio epidemiológico del primer caso diagnosticado en Colombia», *An. Soc. Biol. Bogotá,* **1**: 161-168, 1944.

68. Gatti, C., Boggino, J., et Prieto, C. — «Un nouveau foyer de leishmaniose viscérale en Amérique du Sud», *Bull. Soc. Path. Exot.,* **32**: 602-605. 1939.

69. Guimarães, N. — *Considerações sobre a leishmaniose visceral americana e classificação das leishmanioses.* I Reunião Anual Dermat. Sifil. Brasil, Rio de Janeiro, 1945.

70. Guimarães, N., e Bustamante, F. M. — «A aplicação domiciliária de DDT como base da profilaxia das leishmanioses. Estudo de um foco de leishmaniose mucocutânea cinco anos depois da aspersão periódica com aquele insecticida», *Rev. Brasil. Malariol. & D. Trop.,* **6**: 127-130, 1954.

71. Heisch, R. — «The isolation of *Leishmania* from a ground squirrel in Kenya», *East African Med. J.,* **34**: 183, 1957.

72. Hertig, M., Fairchild, G. B., and Johnson, C. M. — «Leishmaniosis transmission-reservoir project», *Ann. Report Gorgas Mem. Lab.,* **1956**: 9, 1957.

73. Inda, F. F., Vivoli, D., y Vaccarezza, A. J. — «Estudio anatomoclínico de un caso de kala-azar en el adulto», *Semana Méd.,* **41**: 413-424, 1934.

74. Lizarraga, J., y Gulacsy, Z. — «Seis casos de kala-azar infantil en el Pabellon de niños «Luiza Caceres de Arismandi», de Valencia», *II Jornadas Nac. Pueric. y Ped.,* Venezuela, **2**: 693-711, 1955.

75. Lopes, J. A. S. — «Infecção natural de cães por *Leishmania donovani* na cidade de Jacobina, estado da Baía», *Rev. Med. Paraná,* **25**: 57, 1956.
76. Lopes, J. A. S. — «*Phlebotomus longipalpis* naturalmente infestados com formas em leptomonas na cidade de Jacobina, estado da Baía», *Rev. Méd. Paraná,* **25**: 57-58, 1956*a*.
77. Lopes, J. A. S., e Sarno, P. — «Leishmaniose visceral canina em Jacobina, estado da Baía, Brasil», *Bol. Fundação Gonçalo Moniz,* n.° 11, Salvador, Baía, 1956.
78. Maciel, P., e Rosenfeld, G. — «Leishmaniose visceral americana. Um caso de um novo foco», *Rev. Clín. S. Paulo,* **21**: 51-61, 1947.
79. Mangabeira F.°, O. — «11.ª Contribuição ao estudo dos flebótomos. *Phlebotomus oswaldoi* Mangabeira, 1942», *Mem. Inst. Osw. Cruz,* **37**: 287-295, 1942.
80. Martinez, A., y Pons, A. R. — «Primer caso de kala-azar en Venezuela», *Gac. Méd. Caracas,* **48**: 329-332, 1941.
81. Martins, A. V., Brener, Z., Mourão, O. G., Lima, M. M., Sousa, M. A., e Silva, J. E. — «Calazar autóctone em Minas Gerais», *Rev. Brasil. Malariol. & D. Trop.,* **8**: 555-563, 1956.
82. Mazza, S., y Cornejo, J. — «Primeros casos autóctones de kala-azar infantil comprobados en el Norte de la República», *Bol. Inst. Clín. Quirurg.,* Buenos Aires, **2**: 140, 1926
83. Meira, J. A., Jamra, M., e Lima, M. L. M. T. — «Leishmaniose visceral americana. Considerações clínicas, hematológicas e anátomo-patológicas a propósito de um caso», *Arq. Fac. Hig. & Saúde Públ. Univ. S. Paulo,* **2**: 253-300, 1948.
84. Mesnil, F., et Brimont, E. — «Sur un hématozoaire nouveau (*Endotrypanum* n. gen.) d'un édenté de la Guyane», *C. R. Soc. Biol.,* **65**: 581, 1908.
85. Migone, L. E. — «Un cas de kala-azar à Asunción (Paraguay)», *Bull. Soc. Path. Exot.,* **6**: 118-120, 1913.
86. Misle Peña, P., Ron Pedrique, M., y Henriquez, E. — «El tercer caso de kala-azar descrito en Venezuela», *Gac. Méd. Caracas,* **54**: 44-48, 1946.
87. Napier, L. E. — *The principles and practice of Tropical Medicine.* MacMillan Co., New York, 917 pp., 1946.
88. Nussenzweig, V. — «Reacção de fixação do complemento para leishmaniose visceral com antígeno extraído de bacilo da tuberculose. Técnica, sensibilidade e especificidade», *O Hospital,* **51**: 115-127, 1957.
89. Nussenzweig, V. — *Contribuição para o estudo da reacção de fixação do complemento na leishmaniose visceral com antígeno extraído de bacilos de tuberculose.* Tese de doutoramento, Fac. Med. Univ. S. Paulo, 116 pp., 1957*a*.
90. Nussenzweig, V., Nussenzweig, R. S., e Alencar, J. E. — «Leishmaniose visceral canina: reacção da fixação do complemento com antígeno extraído do bacilo da tuberculose», *O Hospital,* **51**: 325-332, 1957.
91. Nussenzweig, V., Nussenzweig, R. S., e Alencar, J. E. — «Leishmaniose visceral canina nos arredores de Fortaleza, estado do Ceará: inquérito sorológico utilizando a reacção de fixação do complemento com antígeno extraído

do bacilo da tuberculose. Observações sobre o diagnóstico e a epidemiologia da doença», *O Hospital,* **52**: 107-129, 1957*a*.

92. Oliveira, A. C. — «Um caso de leishmaniose visceral americana», *O Hospital,* **13**: 465-470, 1938.
93. Pará, M. — Comunicação pessoal ao autor, 1955.
94. Paraense, L., e Chagas, A. W. — «Transmissão experimental da leishmaniose visceral pelo *Phlebotomus intermedius*», *Brasil Méd.,* **54**: 179, 1940.
95. Pena, H. A. — «Leishmaniose visceral no Brasil», *Brasil Méd.,* **48**: 949-950. 1934.
96. Pessoa, S. B. — «Surto epidémico de calazar no município de Sobral (estado do Ceará)», *Rev. Paulista Med.,* **44**: 141-142, 1954.
97. Pessoa, S. B., Silva, L. H. P., e Figueiredo, J. — «Calazar endémico em Jacobina (estado da Baía)», *Bol. Fundação Gonçalo Moniz,* n.º 7, Salvador, Baía, 1956.
98. Pifano, F. — «Notas sobre entomologia médica venezolana. I. Flebotomus transmissores de leishmaniasis tegumentaria en Valle del Yracuy», *Bol. Entomol. Venezol.,* **2**: 99-102, 1943.
99. Pifano, F. — «Estado actual del kala-azar en Venezuela», *Arch. Venezol. Pat. Trop. & Parasit. Med.,* **2**: 213-219, 1954.
100. Pondé, R., Mangabeira F.º, O., e Jansen, G. — «Alguns dados sobre a leishmaniose visceral americana e a doença de Chagas no Nordeste brasileiro», *Mem. Inst. Osw. Cruz,* **37**: 333-352, 1942.
101. Potenza, L., y Anduze, P. J. — «Kala-azar en el Estado de Bolivar, Venezuela», *Rev. Policlín. Caracas,* **11**: 312-317, 1942.
102. Potenza, L., y Barnola, J. — «Leishmaniasis visceral infantil en Venezuela», *Arch. Venezol. Pueric. & Ped.,* **13**: 5-16, 1950.
103. Prata, A. — *Estudo clínico e laboratorial do calazar.* Tese de docência-livre, Fac. Med. Univ. Baía, Brasil, 244 pp., 1917.
104. Prata, A., e Domingues, A. — «Leishmanióide dérmico», *O Hospital,* **50**: 541-558 1956.
105. Prata, A., e Piva, N. — «Presença de leishmânias em pele aparentemente normal de pacientes com calazar», *O Hospital,* **49**: 481-486, 1956.
106. Romaña, C. — «Leishmaniose visceral americana. VIII. Leishmaniasis visceral en la República Argentina», *in* Chagas, Cunha, Castro Ferreira e Romaña, 1937.
107. Romaña, C., Torres, A., y Klappenbach, E. — «Hallazgo de un foco de kala-azar infantil en el Chaco (nota previa)», *La Semana Méd.,* **43**: 954, 1936.
108. Silva, J. Rodrigues — *Leishmaniose visceral (kala-azar).* Tese de cátedra D. Trop. e Infect., Fac. Nac. Med. Univ. Brasil, Rio de Janeiro, 498 pp., 1957.
109. Tavares, A. — «Leishmaniose visceral com diagnóstico positivo, *in vivo,* em Pernambuco», *Impr. Méd.,* **20**: 53-55, 1945.
110. Tavares, A., e Fonte, J. — «Sobre o primeiro caso de leishmaniose visceral com verificação parasitológica em Pernambuco», *Lab. Clín.,* **22**: 133, 1942.

111. Torres, M. — «Alterações cutâneas do cão no kala-azar sul-americano», *Mem. Inst. Osw. Cruz,* **36**: 37-67, 1941.

112. Veronesi, R., Castro, R. M., Marques, J. C., Fiorillo, A. M., Zucolloto, M., Czapski, J., Salles, H. L. B., e Amato Neto, V. — «Leishmaniose visceral (calazar) no Brasil. Estudo do quadro clínico e humoral de 15 novos casos», *Rev. Hosp. Clínicas (S. Paulo),* **10**: 86-111, 1955.

113. Veronesi, R., Jamra, M., Silva, O. R. S., Cruz, O., e Fiorillo, A. — «Leishmaniose visceral (calazar). Estudo do quadro clínico, hematológico e electroforético», *Rev. Hosp. Clínicas (S. Paulo),* **9**: 13-50, 1954.

114. Versiani, O. — «Leishmaniose visceral americana», *Brasil Méd.,* **57**: 268-271, 1943.

115. Villaseñor, J. P., Ruiloba, E. J., Rojas, E., Treviño, A., y Campillo, C.— «Un caso de kala-azar en Méjico», *Bol. Of. Sanit. Panamer.,* **34**: 23-30, 1953.

116. Vivoli, D. — «Anatomía patológica de un nuevo caso de kala-azar americano», *Arch. Soc. Argent. Anat. Normal & Patol.,* **4**: 133-138, 1942.

117. Winckel, W. E. F., and Aalstein, M. — «Contribution to the geographical pathology of Surinam. First case of kala-azar in Surinam», *Docum. Med. Geograph. & Trop.,* **5**: 339-342, 1953.

# DIAGNÓSTICO SOROLÓGICO DA LEISHMANIOSE VISCERAL HUMANA E CANINA[1]

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Provas imunológicas, e em particular as reacções de aglutinação e de fixação do complemento (RFC), têm sido empregadas com certa frequência para o estudo da composição antigénica das espécies que compõem o género *Leishmania*. Poucos, porém, se têm preocupado com o diagnóstico sorológico das leishmanioses, e em particular da leishmaniose visceral. A facilidade com que os parasitas podem ser demonstrados nesta doença, a simplicidade de execução das chamadas reacções sorológicas «inespecíficas», em contraste com as maiores dificuldades técnicas envolvidas em provas imunológicas, certamente contribuíram para que estas últimas fossem relegadas a plano muito secundário na prática. A pesquisa de anticorpos nos soros de doentes de calazar apresenta, no entanto, certas vantagens para o diagnóstico da doença. Permite ainda um melhor *contrôle* de cura e, o que é mais importante, pode contribuir para o esclarecimento de sua patologia e epidemiologia. Na leishmaniose canina, em que os métodos directos de diagnóstico são falhos, o estudo dos métodos sorológicos específicos apresenta particular interesse.

Entre os métodos sorológicos específicos sòmente a RFC tem sido usada com fins diagnósticos. Primitivamente os antígenos empregados eram extraídos de órgãos de animais parasitados ou de formas culturais da *Leishmania donovani*. Anticorpos fixadores foram demonstrados com esses antígenos em soros de animais imunizados e em poucos casos de infecção natural. Primitivamente não se acreditava,

[1] Presented *in absentia*.

porém, na possibilidade de aplicação prática desses antígenos, pois a maioria dos autores que os empregaram encontraram ou resultados negativos ou diminuta sensibilidade em soros de calazar (35). Contudo, no caso de antígenos extraídos de órgãos de animais infectados, pesquisadores chineses (4, 36) utilizaram-nos em larga escala para o diagnóstico do calazar. Os resultados que referem são relativamente bons: 177 reacções positivas em 202 casos da doença e raras reacções inespecíficas. A capacidade fixadora do antígeno estaria na dependência da riqueza em leishmânias do órgão usado na extracção. O antígeno, uma vez preparado, perderia gradualmente o poder fixador. Antígenos desse tipo apresentam, porém, sérios inconvenientes. Sua composição é forçosamente complexa e inconstante. Se a potência do antígeno depende do grau de parasitismo do órgão, poderia ser difícil obter antígenos adequados, pelo menos em curto prazo. Esses inconvenientes, associados à já mencionada instabilidade, tornam seu emprego pouco prático.

Quanto aos antígenos extraídos de formas culturais da *L. donovani,* só também mais recentemente foram empregados em maior número de casos de calazar e com resultados satisfatórios (10, 11, 12). A sensibilidade e especificidade referidas foram excelentes e em alguns doentes a reacção foi precocemente positiva. Recentemente, com antígeno desse tipo, foi demonstrada a presença de anticorpos fixadores de complemento no soro de 44 casos de leishmaniose dérmica pós-calazar (27).

O tipo de antígeno que tem sido de facto mais usado para a pesquisa de anticorpos em soros de calazar é, seguramente, aquele extraído de bacilos acidorresistentes. É desse tipo de antígeno, com que temos trabalhado há alguns anos, que nos ocuparemos mais detidamente neste relatório.

## HISTÓRICO

Os estudos sobre a possibilidade de utilização de antígenos de bacilos acidorresistentes no diagnóstico das leishmanioses tiveram como ponto de partida observações de pesquisadores brasileiros que estudavam a RFC na lepra. Procurando verificar a especificidade dos antígenos usados em moléstias cutâneas que podiam ser confundidas com a lepra, notaram que grande proporção de soros de casos de

leishmaniose tegumentar com eles reagiam (1, 2). Foi mesmo sugerida logo depois a possibilidade de esses antígenos servirem para o diagnóstico da leishmaniose (26), mas os resultados medíocres obtidos em seguida (7), bem como a existência de reacção intradérmica de valor para o diagnóstico daquela afecção cutânea, fizeram com que diminuísse o interesse prático no estudo da RFC.

Enquanto isso, na Índia verificou-se que antígenos preparados de bacilo de tuberculose segundo a técnica de Witebsky, Klingenstein e Kuhn (WKK) (37) reagiam com soros de calazar e que a intensidade da reacção era até mais intensa que nos casos de lepra lepromatosa (14, 16). A utilização dessa reacção para o diagnóstico do calazar foi logo sugerida (15). A capacidade fixadora de complemento era de tal ordem que, na técnica recomendada, o soro suspeito era diluído desde o início a 1/25, e deste modo aumentada a especificidade da prova, mantendo ainda em bom nível a sensibilidade. Os resultados iniciais, com antígeno WKK (13), foram muito bons (31). A partir de 1943, porém, os pesquisadores hindus abandonaram o emprego do bacilo de tuberculose para o preparo do antígeno. Havia, pouco antes, sido demonstrado que reacções semelhantes às obtidas com o antígeno WKK original podiam ser também obtidas com antígenos extraídos de outros bacilos acidorresistentes não patogénicos e de crescimento rápido e exuberante em meios de cultura (5). Usando então antígeno extraído do bacilo de Kedrowsky (6), foram obtidos óptimos resultados numa série grande de casos de calazar (32). Desde então, com técnica pouco diferente da primitiva (33), esse antígeno vem sendo usado rotineiramente na Escola de Medicina Tropical de Calcutá e já é grande a experiência acumulada (39). Outros antígenos, com propriedades semelhantes, têm sido recentemente estudados (18, 19).

Essa série de trabalhos, confirmados em outros países, veio abrir perspectivas novas no estudo da imunidade e diagnóstico do calazar. Na Espanha, em poucos casos da doença foi empregado antígeno extraído do bacilo de tuberculose com álcool metílico (9, 17, 28, 29, 30). No Japão, o *M. tuberculosis avium* foi usado para a extracção do antígeno (20). Mais recentemente estudámos outros aspectos da sorologia do calazar com antígenos aquosos de bacilos de tuberculose prèviamente tratados com acetona (21, 22, 23, 24) e os resultados que obtivemos serão relatados adiante.

## VALOR CLÍNICO DA RFC

*a*) SENSIBILIDADE. — No quadro 1 incluímos alguns dados sobre a sensibilidade da RFC obtidos usando antígenos extraídos de diversas maneiras de bacilos acidorresistentes. Só incluímos os dados que se basearam em casos da doença comprovados pelo encontro do parasita. Além de a sensibilidade da reacção ser muito grande, a reactividade dos soros é geralmente intensa. Utilizando técnica quantitativa em soros de 91 casos de calazar não tratados, verificámos (22) que em cerca de 70 % o título foi maior que 100. A mediana dos títulos foi 188,7 e em vários soros o título foi maior que 1000. Esses níveis de reactividade são bem maiores que aqueles habitualmente encontrados na tuberculose e mesmo na lepra usando antígeno e técnica semelhantes.

QUADRO 1 — *RFC com antígeno de tuberculose em casos de leishmaniose visceral parasitológicamente comprovados, segundo diversos autores*

| Autores | Número de casos | Positivos | | Duvidosos | | Negativos | | Anticomplementares | |
|---|---|---|---|---|---|---|---|---|---|
| | | Número | Percentagem | Número | Percentagem | Número | Percentagem | Número | Percentagem |
| Sanchis Bayarri e Marco Ahuir, 1947 | 33 | 31 | 94,0 | — | — | 2 | 6,0 | — | — |
| Nakamizo, 1949 | 47 | 46 | 98,0 | — | — | 1 | 2,0 | — | — |
| Fernandez-Delgado de la Peña e Carmona, 1950 | 14 | 12 | 85,7 | — | — | 2 | 14,3 | — | — |
| Sen Gupta e Adhikari, 1952 | 358 | 298 | 83,2* | 15 | 4,2 | 2 | 0,6 | 43 | 12 |
| Nussenzweig, 1957*b* | 91 | 89 | 97,8 | — | — | 2 | 2,2 | — | — |

* A percentagem de positivos entre os soros em que a reacção não foi anticomplementar seria 94,6 %.

A RFC torna-se positiva poucas semanas após o início da doença (21, 32) e é seguramente mais precoce que a reacção do aldeído. Em 64 casos em que fizemos simultâneamente as duas provas a RFC foi positiva 63 vezes e a reacção do formol-gel sòmente 36. Fazendo a graduatória por ordem crescente dos títulos dos 64 soros e relacionando as duas provas (22), os resultados mostram um nítido aumento do número de reacções de formol-gel positivas em soros de

títulos mais elevados, provenientes dos casos mais avançados da moléstia (quadro 2).

QUADRO 2 — *Títulos da RFC e reacção de formol-gel em 64 casos comprovados de calazar antes de tratamento específico*

| Grupos | Número de casos | Títulos da RFC | | | Número de reacções de formol-gel | |
|---|---|---|---|---|---|---|
| | | Mínimo | Máximo | Mediana | Positivas | Negativas |
| 1.º grupo . . . . . | 16 | Neg. | 88,9 | 30,7 | 3 | 13 |
| 2.º grupo . . . . . | 16 | 88,9 | 217,5 | 118,4 | 9 | 7 |
| 3.º grupo . . . . . | 16 | 239,1 | 535,0 | 350,1 | 9 | 7 |
| 4.º grupo . . . . . | 16 | 535,0 | 2 562,5 | 650,0 | 15 | 1 |

*b*) ESPECIFICIDADE DA RFC. — É bem conhecida a reactividade dos soros de tuberculose, lepra, leishmaniose cutaneomucosa e leishmaniose dérmica pós-calazar com antígenos extraídos de bacilos acidorresistentes. Recentemente assinalámos (22) reacções cruzadas em soros de doentes com forma aguda e crónica da moléstia de Chagas.

Os títulos são, nessas doenças, geralmente muito mais baixos que no calazar. Podem ocorrer títulos mais altos, porém, em casos de lepra lepromatosa e, pelo menos, em alguns soros de leishmaniose tegumentar e forma aguda de doença de Chagas. Essas doenças não se confundem, porém, com as formas avançadas de calazar.

*c*) REACTIVIDADE DO SORO E DADOS CLÍNICOS. — Pudemos correlacionar os títulos da RFC a alguns dados clínicos obtidos de maneira sistematizada em um grupo de doentes de calazar (22). Em grupos de doentes com tempo referido de duração da doença progressivamente maior verificámos que a mediana dos títulos também cresce. Por outro lado, separando todos os casos, independentemente da duração da doença, em dois grupos etários (menos de 3 anos e 3 ou mais anos), notámos também significativa diferença na mediana dos títulos: nas crianças de baixa idade os títulos foram menores. A influência da idade continuou a se fazer sentir mesmo quando foram com-

parados grupos com o mesmo tempo de duração de doença referido. Além disso, os soros de doentes com maior hipertrofia do baço e fígado, de qualquer dos grupos etários, têm maiores títulos, indicando que o grau de reactividade do soro reflecte o desenvolvimento da doença.

*d*) RFC E TRATAMENTO. — O efeito do tratamento específico sobre a reactividade do soro de doentes de calazar tem sido pouco estudado. As técnicas usadas não permitem mesmo avaliação correcta da variação do poder fixador do soro com o tratamento. Quando o acompanhamento sorológico foi feito, mostrou de modo geral uma queda ou mesmo desaparecimento da reactividade do soro (31, 32, 34).

Esse aspecto da sorologia do calazar apresenta interesse especial, pois é conhecida a dificuldade de estabelecer um critério de cura da doença. Procurando esclarecê-lo, fizemos um estudo sistematizado em 82 casos comprovados parasitològicamente. A RFC foi feita por técnica quantitativa em épocas variáveis após o tratamento específico — geralmente uma série de 10 a 20 injecções de N-metil-glucamina, na dose de 100 mg ao dia por quilo de peso. Os resultados estão resumidos no quadro 3.

Como se vê, logo após o término do tratamento pode haver em alguns casos um aumento da reactividade do soro, sem, ao que parece, qualquer significação prognóstica. Geralmente, porém, a queda do título é acentuada, atingindo este, após um mês, 50 % ou menos do valor inicial. Daí por diante a queda é muito mais lenta. A negativação da RFC só se dá muito tempo após a cura clínica.

Verificámos que títulos baixos podem ser encontrados com frequência, na ausência de sintomas, até pelo menos dois anos do término do tratamento. Ora é conhecido o facto de no calazar as recaídas poderem verificar-se mesmo em casos com acentuadas melhoras clínicas após tratamento. Por outro lado, a localização dérmica do parasito muito tempo após a cura das lesões viscerais não é rara em certas zonas endémicas. Esses factos indicam a possibilidade de existir na leishmaniose visceral, com certa frequência, uma dissociação entre a cura clínica e parasitológica.

QUADRO 3 — *Variação do título da RFC com o tratamento antimonial de doentes de calazar parasitològicamente comprovados*

| Número de dias após o tratamento em que foi feita a RFC | Número de casos examinados | Comportamento do título da RFC com o tratamento | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mediana antes do tratamento | Mediana depois do tratamento | Percentagem de queda da mediana | Número de casos em que se elevou ou se manteve | Número de casos com queda do título entre | | | | |
| | | | | | | 5 e 20 % | 21 e 40 % | 41 e 60 % | 61 e 80 % | 81 e 98 % |
| 0 a 10 . . . . . | 46 | 264,0 | 160,3 | 39 | 16 | 8 | 9 | 8 | 5 | 0 |
| 11 a 40 . . . . . | 18 | 188,6 | 73,4 | 61 | 0 | 1 | 2 | 2 | 12 | 1 |
| 41 a 180. . . . . | 18 | 244,5 | 34,1 | 86 | 1 | 0 | 1 | 1 | 5 | 10 |
| Mais de 180 * . . . | 25 | — | 9,9 | — | — | — | — | — | — | — |

* Neste grupo não foi feita a RFC antes do tratamento; inclui doentes nos quais o tratamento fora completado de 6 meses a 2 anos antes da colheita de sangue para a RFC.

## A NATUREZA DA REACÇÃO

O antígeno que reage com soro de doentes de calazar tem sido extraído de diversos bacilos acidorresistentes, patogénicos ou não, e não foi demonstrada qualquer diferença quanto às suas propriedades fixadoras de complemento. Por essa razão, os autores hindus têm dado preferência aos saprófitos como fonte de antígeno, por ser seu cultivo abundante e de crescimento mais rápido. É variável também o modo de extracção do antígeno da massa bacteriana. Está bem estabelecido que a fracção fixadora de complemento é insolúvel em acetona e álcool etílico e solúvel em piridina e benzol. É termoestável: resiste bem à fervura em água.

Sua natureza não é ainda bem conhecida. Sendo a fracção antigénica a mesma que fixa complemento em presença dos soros de lepra e tuberculose, deve tratar-se de um complexo lipopolissacarídico (8, 25). Aliás, o comportamento do antígeno diante do soro de calazar se assemelha ao do mesmo antígeno diante do soro homólogo, bem como, de modo geral, ao de outros sistemas fixadores de complemento (22).

Não vemos razão para considerar inespecífica a reacção do antígeno de tuberculose com o soro de calazar, como sugerem alguns autores. Pelo contrário, vários factos indicam que se trata de verdadeira reacção antígeno-anticorpo. Não há dúvida de que o soro de calazar é particularmente sensível à floculação. Essa labilidade do soro ocorre, porém, sòmente em fases adiantadas da doença e a RFC é positiva muito mais precocemente. É sabido também que outros soros lábeis, em que existe profunda alteração das proteínas plasmáticas, não reagem com o antígeno. A RFC é de grande sensibilidade e pode tornar-se positiva muito precocemente, como sói acontecer com reacções imunológicas. Em certos soros ocorre fenómeno curioso, frequente em outros tipos de provas imunológicas, isto é, uma nítida inibição da fixação do complemento nas maiores concentrações de soro (15, 22, 24). O grau de inibição depende da dose de antígeno presente (22). Antígenos de tuberculose reagem também, como vimos, com soros de outras doenças produzidas por parasitos do género *Leishmania* e *Trypanosoma*. Experiências de absorção de soros de calazar com flagelados do género *Leishmania* e *Trypanosoma* mos-

traram que, pelo menos, parte das substâncias que reagem com o antígeno de tuberculose puderam ser retiradas (22). Acreditamos pois que se trate de fenómeno de imunidade cruzada devido à presença de fracções antigénicas semelhantes ou comuns em bacilos acidorresistentes e nos flagelados citados.

## O DIAGNÓSTICO SOROLÓGICO DA LEISHMANIOSE CANINA

Dois têm sido os métodos preconizados para o diagnóstico da leishmaniose canina: os métodos sorológicos e os directos. Entre os primeiros foi utilizada com maior frequência a reacção do aldeído, certamente pelo valor que ela apresenta para a comprovação da afecção humana. Sua utilidade diagnóstica é, porém, para a maioria dos autores, limitada, servindo, quando positiva, sòmente para o diagnóstico de presunção da doença (3). Quanto aos métodos directos, diferentemente do que ocorre na infecção do homem, têm menor valor, como discutimos em outro trabalho (23). Por isso, aliás, o estudo da evolução clínica da doença em condições naturais, bem como a avaliação do papel desempenhado pelo cão na epidemiologia da doença, são difíceis quando se apoiam apenas no emprego de métodos directos.

Em vista dos resultados obtidos com a RFC com antígeno de tuberculose na afecção humana, foi estudada a possibilidade de seu emprego na leishmaniose canina (3, 23, 24). A RFC é muito sensível: em cães nos quais a doença foi demonstrada parasitològicamente a RFC foi positiva, em geral com títulos muito elevados. A aplicação da RFC em inquéritos em zonas endémicas do Nordeste do Brasil (3, 24) demonstrou que sua sensibilidade é bem maior que a da reacção do aldeído ou que a da pesquisa do parasita por punção do fígado ou pele do animal. Num desses inquéritos, por exemplo, dos 50 cães com RFC positiva 25 tiveram comprovação parasitológica — em 6 deles, porém, o parasita só foi demonstrado depois de repetição das punções nos animais, em vista de resultados sorológicos positivos. Seis meses após, reexaminada uma parte dos animais com RFC positiva e sem comprovação parasitológica, em 4 dentre 11 foram encontradas leishmânias na pele. Ao que parece, a especificidade da RFC é boa, pois a reacção foi negativa em cerca de uma centena de cães de zona onde a doença não é conhecida. Em vista, porém, das reac-

ções cruzadas assinaladas no homem e já referidas, julgamos que esse problema deverá ser melhor estudado.

## BIBLIOGRAFIA


1. Assumpção, L., e Silveira, G. F. — «Contribuição ao estudo da fixação do complemento na lepra», *Rev. Leprol. São Paulo*, **2**: 13-36, 1935.
2. Bier, O., e Planet, N. — «Aplicação do processo de Witebsky ao preparo de um antígeno para fixação do complemento na lepra com o *Streptothrix leproides* (Deicke)», *Folia Clin. Biol.*,, **8**: 72-75, 1936.
3. Brener, Z. — *Calazar canino em Minas Gerais*. Tese, Faculdade de Medicina, Belo Horizonte, 90 pp., 1957.
4. Chung, H. L., and Chang, N. C. — «A kala-azar complement fixation test; its diagnostic and prognostic value», *Chin. Med. J.*, **69**: 3-18, 1951.
5. Dharmendra and Bose, R. — «Complement-fixation in leprosy with antigens prepared from various acid-fast bacilli», *Indian J. Med. Res.*, **29**: 7-21, 1941.
6. Dharmendra, Bose, R., and Sen Gupta, P. C. — «The preparation of an antigen from the Kedrowsky's bacillus for the complement-fixation test for kala-azar», *Indian J. Med., Res.*, **34**: 1-2, 1946.
7. Eichbaum, F. W. — «Fixação do complemento com o antígeno de Witebsky-Kuhn-Klingenstein em dermatoses tropicais: lepra, leishmaniose, pênfigo foliáceo e blastomicose», *Rev. Brasil. Biol.*, **2**: 285-300, 1942.
8. Faure, M. — *Les haptènes de fixation de l'alexine. Recherches sur l'haptène lipoïdique et les phosphatides du bacille tuberculeux*. Publicação do Instituto Pasteur, Paris, 230 pp., 1940.
9. Fernández Delgado de la Peña, A., y Carmona, M. D. — «Diagnóstico serológico del kala-azar», *Acta Pediatr. Esp.*, **8**: 699-704, 1950.
10. Ghosh, H., and Ghosh, N. N. — «Complement-fixation reaction in sera of rabbits actively immunized with living culture of *Leishmania donovani*», *Ann. Biochem. Exp. Med.*, **7**: 1-2, 1947.
11. Ghosh, H., Ghosh, N. N., and Ray, J. C. — «Preliminary note on the complement-fixation reaction in kala-azar with specific antigen as an aid to diagnosis», *Ann. Biochem. Exp. Med.*, **5**: 153-158, 1945. Resumo in *Trop. Dis.. Bull.*, **43**: 1125-1126, 1946.
12. Ghosh, H., Ghosh, N. N., and Ray, J. C. — «Further studies on complement-fixation reaction in kala-azar with a specific antigen as an aid to diagnosis», *Ann. Biochem. Exp. Med.*, **9**: 173-178, 1949.
13. Greval, S. D. S., Das, B. C., and Sen Gupta, P. C. — «Preparation and use of the Witebsky. Klingenstein and Kuhn (W.K.K.) antigen», *Indian J. Med. Res.*, **29**: 527-530, 1941.
14. Greval, S. D. S., Lowe, J., and Bose, R. — «Complement-fixation in leprosy with Witebsky, Klingenstein and Kuhn (W.K.K.) antigen: a new technique», *Indian J. Med. Res.*, **26**: 843-849, 1939.

15. Greval, S. D. S., Sen Gupta, P. C., and Napier, L. E. — «Serological reactions in kala-azar: complement fixation, false Wassermann reaction, and high anti-complementary titre», *Indian J. Med. Res.*, **27**: 181-190, 1939.
16. Lowe, J., and Greval, S. D. S. — «Complement-fixation in leprosy and other diseases by the Witebsky, Klingenstein and Kuhn (W.K.K.) antigen», *Indian J. Med. Res.*, **26**: 833-841, 1939.
17. Marco Ahuir, R., y Selfa, J. — «Diagnóstico exclusivamente sorológico de la leishmaniosis visceral infantil», *Rev. Esp. Pediat.*, **9**: 55-59, 1953.
18. Monsur, K. A. — «Alcoholic extracts of Kedrowsky's bacillus as antigen for complement-fixation tests in kala-azar», *Trans. Roy. Soc. Trop. Med. & Hyg.*, **50**: 91-96, 1956.
19. Monsur, K. A., e Khaleque, K. A. — «Preparation of a purified antigen from Kedrowsky's bacillus for complement-fixation test for kala-azar», *Trans. Roy. Soc. Trop. Med. & Hyg.*, **51**: 527-532, 1957.
20. Nakamizo, J. — «O diagnóstico sorológico do calazar usando antígeno extraído do *Mycobacterium tuberculosis avium*» (em japonês), *Boletim da Associação Médica do Exército Japonês*, **363**: 895-903, 1949.
21. Nussenzweig, V. — «Reacção de fixação do complemento para leishmaniose visceral com antígeno extraído do bacilo da tuberculose. Técnica, sensibilidade e especificidade», *O Hospital*, **51**: 217-226, 1957*a*.
22. Nussenzweig, V. — *Contribuição para o estudo da reacção de fixação de complemento na leishmaniose visceral com antígeno extraído de bacilos de tuberculose*. Tese, Faculdade de Medicina da Universidade de S. Paulo, 116 pp., 1957*b*.
23. Nussenzweig, V., Nussenzweig, R. S., e Alencar, J. E. — «Leishmaniose visceral canina: reacção de fixação de complemento com antígeno extraído do bacilo da tuberculose», *O Hospital*, **51**: 325-332, 1957*a*.
24. Nussenzweig, V., Nussenzweig, R. S., e Alencar, J. E. — «Leishmaniose visceral canina nos arredores de Fortaleza, estado do Ceará: inquérito sorológico utilizando a reacção de fixação do complemento com antígeno extraído do bacilo de tuberculose. Observações sobre o diagnóstico e epidemiologia da doença», *O Hospital*, **52**: 111-129, 1957*b*.
25. Pangborn, M. C. — «Studies of tuberculosis. Antigens of the tubercle bacillus», *Annual report of the division of laboratories and research, New York State Department of Health*, 11-12, 1956.
26. Rabelo Jr., Pinto, J. T., e Tostes, J. — «Reacção de Witebsky na leishmaniose», *Anais Brasileiros de Dermatologia e Sifiligrafia*, **14**: 217-218, 1939.
27. Roy, A. N., Banerjee, G., and Banerjee, B. N. — «A preliminary investigation on the complement-fixation reaction in dermal leishmaniasis», *Ann. Biochem. Exp. Med.*, **51**: 109-112, 1955.
28. Sanchis Bayarri, V., y Marco Ahuir, R. — «Contribución al suerodiagnóstico del kala-azar con el empleo del antígeno metílico tuberculoso», *Rev. Esp. Pediatr.*, **3**: 773-780, 1947*a*.

29. Sanchis Bayarri, V., y Marco Ahuir, R. — «Diagnóstico serológico del kala-azar con el antígeno metílico tuberculoso», *Rev. Clín. Esp.*, **25**: 103-107, 1947*b*.
30. Sanchis Bayarri, V., y Marco Ahuir, R. — «Sobre el suerodiagnóstico del kala-azar con el antígeno metílico tuberculoso», *Med. Colon.*, **12**: 279-282, 1948. Resumo in *Trop. Dis. Bull.* **46**: 127, 1949.
31. Sen Gupta, P. C. — «A complement-fixation test for kala-azar», *Indian Med. Gaz.*, **78**: 336-339, 1943.
32. Sen Gupta, P. C. — «The value of the complement-fixation test in the diagnosis of kala-azar», *Indian Med. Gaz.*, **79**: 465-469, 1944.
33. Sen Gupta, P. C. «Complement-fixation test with Witebsky, Klingenstein, Kuhn (W.K.K.) or similar antigens: a modified technique», *Indian Med. Gaz.*, **80**: 396-398, 1945.
34. Sen Gupta, P. C., and Adhikari, S. L. — «Observations on the complement-fixation test for kala-azar», *J. Indian Med. Assoc.*, **23**: 89-93, 1952.
35. Taliaferro, W. H. — *The immunology of parasitic infections*. New York, London, The Century Co., 414 pp., 1929.
36. Weng, H. C., Chung, H. L., Hou, T. C., and Ho, L. Y. — «A simplified antigen for kala-azar complement-fixation test with observations in 742 cases», *Chin. Med. J.*, **71**: 328-333, 1953.
37. Witebsky, E., Klingenstein, R., e Kuhn, H. — «Serodiagnostiche Untersuchungen bei Tuberkulose», *Klin. Wschr.*, **10**: 1068-1071, 1931.

# THE TRANSMISSION OF CUTANEOUS LEISHMANIASIS IN VENEZUELA BY *P. PANAMENSIS* SHANNON 1926

FELIX PIFANO

In collaboration of Prof. J. V. Scorza, Br. I. Ortiz,
Sr. A. Alvarez y Dr. C. Dager

Since 1940 epidemiological evidence has accumulated about the role of *P. panamensis* in the transmission of cutaneous leishmaniasis in Venezuela.

In an area of high prevalence we demonstrated a 6.94 % of natural infection of *P. panamensis* with leptomonae forms. Total specimens examined were 72.

*P. panamensis* is widely distributed in forests. It penetrates hamlets and it is highy anthropophilic. We collected some females biting the edges of active lesions of leishmaniasis. In the stomach of these specimens, four days later, we discovered the presence of leptomonae forms.

We have also found two other species of *Phlebotomus, P. migonei* and *P. longipalpis,* naturally infected.

With the aim of studying the anthropophilic habits of *Phlebotomus* of this area, we used a healthy person as bait. We collected the *Phlebotomus* with specimen tubes as soon as they were engorged, without damaging them. The insects were taken to the laboratory in order to classify the females after oviposition.

In this experiment we collected 17 specimens, of *P. panamensis* only.

Four weeks later, the healthy man who had been used as bait developed two nodules, one on the back of the right arm and other on the anterior part of the right leg. Eight weeks after biting by the insects, the nodules ulcerated and the clinical characteristics were

typical of cutaneous leishmaniasis. In these lesions *Leishmania* were found in touch smears.

We did not dissect the *Phlebotomus* because the purpose of our experiment was to study the biting habits of the insects.

These findings are one step more in demonstrating the transmission of cutaneous leishmaniasis by *Phlebotomus* in America, and in our opinion of *P. panamensis* as a vector in Venezuela.

## GENERAL DISCUSSION

Dr. P. Giraud (France) — L'auteur rend hommage aux travaux de Monsieur Blanc qui ont orienté les travaux épidémiologiques qu'il a poursuivis à Marseille depuis 35 ans.

Il a observé les piqûres des tiques chez de jeunes enfants. Cet arthropode est d'ailleurs l'agent vecteur de la fièvre butoneuse-méditerranéenne, assez fréquente chez l'enfant dans cette région.

Enfin, il insiste sur la nécessité de ne pas se contenter des diagnostics hématologiques et sérologiques pour le diagnostic du kala-azar. La mise en évidence directe ou par culture du parasite, souvent de technique difficile, doit être considérée comme la seule méthode diagnostique de certitude.

Dr. A. Packchanian (U.S.A.) — The last speaker stated that *Leishmania donovani* was transmitted to man by the bite of the ticks. I would like to know the taxonomic name of this tick. Were the parasites in the ticks flagellar or aflagellar forms and have you been able to transmit infection to the experimental animals by the bite of the ticks?

On this occasion I would like to mention that a few years ago I produced experimental visceral leishmaniasis in several species of American rodents, i. e., *Dipodomys, Peromyscus maniculatus gambeli, Peromyscus californicus, P. polinotus,* etc. (unpublished data). These findings gave us a few additional species of laboratory animals for studying experimental leishmaniasis and suggest the potential possibility that several species of American rodents may serve as reservoir hosts for *Leishmania.*

Dr. Rafael Medina (Venezuela) — Quiero hacer referencia a lo necesario que es el estudio de dos especies de leishmanias en el continente americano, por cuanto algunas cepas presentan un comportamiento bastante diferente. Nosotros en Venezuela hemos tenido

oportunidad de estudiar por ejemplo una (que denominamos Pifanvi). Once enfermos han sido estudiados hasta el presente: 6 con la forma generalizada y 5 con la forma cutánea de lesión única. Las formas cutáneas no ceden con la terapia habitual, evolucionan por brotes, no invaden la mucosa nasal y nunca atacan las vísceras. La riqueza de parasitas es notable: 200-300 por campo. La infección del *hamster* se logra en el 100 % de los casos (a diferencia de la cepa *brasiliensis* clásica); esta infección es puramente cutánea, sin ocurrir la generalización. Considero, en fin, esta cepa como absolutamente distinta de la especie *L. brasiliensis* Vianna, 1911, y pungo a disposición de los colegas (especialmente los de países tropicales) la cepa en referencia.

Dr. M. H. Sati (Sudan) — I should like to ask the last speaker the following:

1. What form of antimony he used in Venezuela? and
2. Whether he used the diamidines.

Dr. Felix Pifano (Venezuela) — Existen diferencias clínicas entre leishmaniasis *tegumentaris* clásica (endemias en Norte Argentina, Bolivia, Perú, Ecuador, Colombia y Venezuela) y la leishmaniasis de Centro América.

Dr. P. Giraud (France) — La tique qui est très répandue dans notre région est *Rhipicephalus sanguineus*. La preuve du rôle possible de cet arthropode a été fournie par Mr. Blanc, qui a infecté des tiques par piqûre de l'animal infecté.

Ces tiques ont conféré la maladie par injection de leur broyat à l'animal sensible même après un temps assez long.

Dr. J. Alencar (Brasil) — O calazar é prevalente no Brasil nos estados do Ceará e Piauí, onde se apresenta em estado endémico. Em outros estados do Nordeste e Leste apresenta-se sob a forma esporádica.

Há uma coincidência geográfica entre a doença canina e a doença humana, mas a primeira tem uma incidência maior, se bem que variável de estado para estado e de ano para ano, de acordo com a marcha do trabalho profiláctico. Nos estados do Ceará e Piauí, por exemplo, no Nordeste, onde há uma endemia, tivemos de 1953 para cá os

seguintes casos: 140, 362, 434, 347, 309; total, 1592 casos. No País inteiro tivemos cerca de 2200 casos desde o início da descoberta da doença por Migone, em 1913.

Há também uma coincidência geográfica da moléstia, não sòmente canina, como humana, com a do *P. longipalpis*. Entretanto devemos ver que há regiões em que existe a doença canina e não a humana, como verifiquei num dos bairros da cidade de Fortaleza, com alta densidade de cães doentes e nenhum caso humano. Mas aí verifica-se que houve uma dedetização ampla para profilaxia da malária, e por isso não há flebótomos. Verifica-se então um mecanismo de transmissão entre cães que dispensa o flebótomo. Vale informar que os cães aí são muito parasitados por *Rhipicephalus sanguineus* e têm o hábito de comer os seus carrapatos, como também os de outros cães.

Em 163 305 flebótomos estudados durante 4 anos verificámos que 95 % pertencem à espécie *P. longipalpis*. Essa percentagem, também encontrada por Deane, aplica-se às outras áreas do País onde há calazar.

Novos focos de calazar no Brasil estão-se instalando ao longo das estradas de emigração interna de populações do nordeste para o sul; os emigrantes carregam consigo os cães, que, quando doentes, instalam novos focos da doença onde há *P. longipalpis*.

Temos usado para a profilaxia da moléstia o DDT intradomiciliar e nos abrigos de animais domésticos, na base de 1,50 g por metro quadrado de parede. Ao lado dessa medida estamos fazendo inquéritos caninos, examinando todos os cães da região, por punção hepática e esfregaço de pele da orelha. Após eliminamos os cães doentes, mas nas regiões endémicas estamos tentando a *descanização*, isto é, a eliminação completa dos cães, medida de aplicação difícil, mas que dará bons resultados.

A dedetização por si só não elimina a moléstia humana, pois há uma pequena quota de contágio extradomiciliar, como também a diminuição dos cães domésticos não determina a erradicação, pois, como demonstrou Deane (1954), há um reservatório silvestre, a raposa *(Lycalopex vetulus)*, que manterá com certeza em certas zonas casos esporádicos.

Dr. Georges Blanc (Maroc) — Le Dr. Blanc rappelle que non seulement il est possible d'infecter expérimentalement des *Rhipicepha-*

*lus,* mais que ceux-ci restent infectés après les différentes mues et que *Rickettsia burneti* peut se conserver au moins un an chez les tiques mortes.

Dr. Samuel Pessoa (Brasil) — Envia a sugestão para que a Organização (Oficina) Sanitária Panamericana se torne o órgão coordenador dos estudos sobre leishmanioses na América.

Dr. Cecilio Romaña (Argentina) — El Dr. Romaña sugerió la conveniencia de realizar un programa combinado para dilucidar las relaciones existentes entre las diversas cepas de leishmanias en América y en el antiguo continente y que este trabajo se confie a Venezuela o al Instituto Pasteur de Paris.

Dr. S. Adler (Israel) — Proposes that this Congress stresses the necessity of international cooperation in the study of leishmaniasis. It is desirable that strains from different endemic foci be made available to research workers in all interested laboratories. It is further desirable that strains be maintained in one or more central laboratories.

3RD SESSION: **Symposium on chemotherapy of leishmaniasis**

*Tuesday, September 9th — 11:00 to 12:30 a.m. Room C*

The session was opened by Dr. Saul Adler, Chairman of the meeting, and the Rapporteur was Dr. Samuel Pessoa. The Secretary was Dr. Maria do Pilar F. Gomes.

The following papers were presented and discussed.

# CHEMOTHERAPY OF EXPERIMENTAL LEISHMANIASIS [1]

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## INTRODUCTION

Most published procedures for the *in vivo* testing of compounds for activity against the *Leishmania* involve considerable effort and time, often as much as two months for completion of a trial. Our recently published method, which takes only eight days to complete, has been shown to be both sensitive and economical (1, 2). Two objections may be raised to the method as published: 1) that some slow-acting compounds may not be given an adequate trial in so short a period of time and 2) that *in vivo* testing in the hamster alone, because of that animal's peculiar sensitivity to or metabolism of particular chemical compounds, may fail to reveal compounds of

[1] This investigation was supported by a research grant (E-92) from the Institute of Allergy and Infectious Diseases, Public Health Service.

[2] The invaluable technical assistance of Elizabeth M. Franchino, Paul Actor, John Grun and Richard Rossan is gratefully acknowledged.

potential usefulness. This report deals with attempts to evaluate these objections. It also includes the results from trials with a few types of compounds not previously tested by us.

## MATERIALS AND METHODS

The Khartoum strain of *Leishmania donovani* produces highly characteristic infections upon the intravascular introduction of known numbers of parasites (3). The early courses of these infections are so uniform that they lend themselves to statistical treatment. Relatively small deviations may, therefore, be statistically significant. These studies have been made in a number of species of experimental animals (4, 5).

The host animals used in the present study are the golden hamster *(Cricetus auratus)*, the albino mouse *(Mus musculus)*, the cotton rat *(Sigmodon h. hispidus)*, and the chinchilla *(Chinchilla lanigera)*.

All animals were infected by injection, either intravenously or intracardially, with saline suspensions of heavily-infected hamster spleens ground in a Ten Broeck glass tissue grinder. Estimates of the numbers of parasites injected were made as previously described (2, 3, 5) and confirmed, in every experiment, by sacrificing one group of animals at 1 or 2 hours after inoculation. Approximately 5 million parasites are usually injected per 100 g of host animal.

At necropsy, liver and spleen weights were recorded and liver and spleen impression smears were made for the counting of parasites. The Giemsa-stained smears were examined and parasite density recorded as parasites per organ cell nucleus. In all instances reported here, 1,000 organ cell nuclei were counted (see Goodwin, 6). The density ratio so obtained multiplied by the milligrams of organ weight (called «total Lds») is a direct function of the actual numbers of parasites in the host animal (3). It is used here to represent that number.

All chemical compounds used in these experiments were injected intraperitoneally in single daily doses beginning 24 hours after inoculation of parasites. Drug treatment continued for 6, or 12, days with all experiments terminating on the 8th ou 14th day of the experiment. Untreated control groups, sacrificed at the end of the exper-

iment, were included and used as the reference for the statistical comparisons.

The compounds used were as follows: *amphotericin B,* and a more soluble form of it known as fungizone, courtesy of Dr. J. Pagano, Squibb Institute for Medical Research, New Brunswick, N. J.; *fumagillin,* courtesy of Dr. G. F. Otto, Abbott Laboratories, North Chicago, Ill.; *pentostam,* courtesy of Dr. D. W. Adamson, Wellcome Research Laboratories, Kent, England; *pentamidine,* courtesy of Dr. F. Goble, Ciba Pharmaceutical Co., Summit, N. J.; *M Sb* and *Mel B,* courtesy of Dr. E. A. H. Friedheim, New York, N. Y.; and *tritheon,* courtesy of Dr. Albert Kupferberg, Ortho Research Foundation, Raritan, N. J.

*Mel B, tritheon* and *fumagillin* were suspended in 2 % gum acacia; the other compounds were administered as saline solutions.

## RESULTS

The results are divided into three sections designed to shed light on the two possible objections mentioned previously and to report the trials with the previously untested compounds.

### A FOURTEEN-DAY METHOD FOR THE HAMSTER.

In this experiment pentamidine and pentostam were compared for both 6 and 12 days of treatment at doses presumed from previous work (2) to be close to the minimum effective levels. Pentamidine is toxic to hamsters at doses so close to the effective level (2) that it may here represent a compound which might have been missed by the 8-day method at the maximum tolerated dose (7). The pentostam, as in previous studies (1, 2), is the reference compound. The results are summarized in Tables 1 and 2.

The results shown in Table 1 confirm the previous report (2) and show that pentostam is active at levels of 47 and 94 mg/kg body weight when administered daily for 6 days. Likewise, pentamidine is active at 12.5 mg/kg. In addition, pentamidine is shown not to produce a statistically significant effect in the course of the infection at the 6.25 kg dosage level.

TABLE 1 — *The effect of pentamidine and pentostam on* L. donovani *infections in hamsters as demonstrated by the 8-day method using liver impression smears for the parasite counts. Each group contained 7 animals*

| Treatment group | Group mean Total Lds | $p$ values [1] |
|---|---|---|
| None — 2 hr. control . . . . . | 39 | — |
| None — 8 day control . . . . . | 1,367 | — |
| Pentostam — 47 mg/kg . . . . . | 382 | $< 0.001$ |
| Pentostam — 94 mg/kg . . . . . | 122 | $< 0.001$ |
| Pentamidine — 6.25 mg/kg . . . . . | 663 | $< 0.2$ or $> 0.1$ not signif. |
| Pentamidine — 12.5 mg/kg . . . . . | 437 | $< 0.001$ |

[1] All $p$ values obtained by comparing data for specified group with the data for the untreated 8-day control group.

TABLE 2 — *The effect of pentamidine and pentostam on* L. donovani *infections in the hamster as determined by a 14-day method using parasite counts from liver impression smears. Each group contained 7 animals*

| Treatment group | Group mean Total Lds | $p$ values [1] |
|---|---|---|
| None — 2 hr. control . . . . . | 39 | — |
| None — 14 day control . . . . . | 6,827 | — |
| Pentostam — 24 mg/kg . . . . . | 5,327 | $< 0.3$ or $> 0.2$ not signif. |
| Pentostam — 47 mg/kg . . . . . | 1,008 | $< 0.001$ |
| Pentamidine — 6.25 mg/kg . . . . . | 2,591 | $< 0.01$ or $> 0.001$ |
| Pentamidine — 12.5 mg/kg . . . . . | 437 | $< 0.05$ |

[1] All $p$ values obtained by comparing data for specified group with the 14-day untreated control group data.

Table 2 shows the results of the 14-day method in this combined experiment. So far as pentostam is concerned, there appears to be no particular advantage of the 14-day over the 8-day method. Again 47 mg produces a significant inhibition of the expected increase of parasite numbers, but the next lower dose (24 mg) is not shown to be active in spite of 12 days of drug administration.

In the case of pentamidine, the 12.5 mg dosage is effective by both methods. At the lower dosage (6.25 mg), however, the extra 6 days of drug administration in the 14-day method does produce a definite inhibition of the expected parasite increase.

It is interesting to point out that, if the results from spleen smears instead of liver smears are the basis for the comparisons, higher doses than indicated are necessary to produce a significant reduction in parasite numbers. For example, in the spleen data from the 8-day portion of the experiment given above only the 94 mg level of pentostam per kilogram gives a significant reduction from the 8-day untreated control value. This is a statistical problem and not surprising since the parasites in the spleen are so few in number in this type of experiment, representing less than 1 % of those in the animal during these time intervals (3).

### Other hosts for the 8-day method.

*Cotton rat.* — In an experiment entirely similar to the 8-day hamster experiments reported here and previously (2) the cotton rat, infected with *L. donovani* and treated with pentostam, showed almost identical effects of the drug. A portion of this experiment is shown in Table 3.

Table 3 — *The effect of pentostam on visceral leishmaniasis in the cotton rat and chinchilla as determined from liver impression smears*

| Treatment group | Group median. Total Lds | |
|---|---|---|
| | Cotton rat | Chinchilla |
| None — 1 hr. control | 16 | 300 * |
| None — 8-day control | 677 | 1,925 |
| Pentostam — 94 mg/kg | 11 | 8 |

* Not done; evidence from other experiments with similar inocula.

It is clearly evident that even as little as 94 mg/kg of drug per day for 6 days is adequate to suppress completely the expected rise in parasite numbers. Other groups in this experiment received 188, 375 and 750 mg/kg. The animals receiving the latter two doses all showed

negative liver smears. At 188 mg, 50 % of the animals in the group showed negative smears.

The spleen counts were essentially similar, even 94 mg/kg showing a distinct suppression of parasites below the level introduced by inoculation. These results confirm the work of Fulton, Joyner and Chandler (8) on the suitability of the cotton rat for chemotherapy screening.

*Chinchilla.* — In a similar experiment with the chinchilla 94 mg/kg of pentostam was again highly active (see Table 3).

*Mouse.* — Although the albino mouse, because of its small size, would seem to be ideal as a host animal in chemotherapeutic trials, its relative insusceptibility to visceral strains of *Leishmania* has apparently prevented its exploitation. As pointed out previously (3, 5), the mouse under the procedures used by us is moderately susceptible to infection with the Khartoum strain of *L. donovani*. This is manifested by a 3 to 5 fold increase in parasites over the first 8 days after intravenous inoculation of parasites. Therefore, the application of the 8-day method of drug screening to the visceral infection in the mouse seemed the next logical step. The experimental plan was the same as that used for cotton rat, hamster and chinchilla. The results are shown in Table 4.

TABLE 4 — *The effect of pentostam on visceral leishmaniasis in the mouse as determined from liver and spleen impression smears. Each group contained 10 animals. All negative smears treated as if 1 parasite had been found*

| Treatment group | Group mean Total Lds in liver | $p$ values [1] | Group mean Total Lds in spleen | $p$ values [1] |
|---|---|---|---|---|
| Untreated — 1 hr. control . . . . . | 114 | — | 0.6 | — |
| Untreated — 8-day control . . . . | 661 | — | 2.7 | — |
| Saline — 8-day control . . . . . | 554 | — | 2.4 | — |
| Pentostam — 94 mg/kg . . . . . | 151 | $<0.1$ | 2.4 | $<0.9$ |
| Pentostam — 188 mg/kg . . . . . | 1.5 | $<0.01$ | 0.6 | $<0.1$ |
| Pentostam — 375 mg/kg . . . . . | 0.2 | $<0.01$ | 0.2 | $<0.05$ |
| Pentostam — 750 mg/kg . . . . . | 0.1 | $<0.01$ | 0.1 | $<0.05$ |

[1] All $p$ values obtained from comparing data from specified group with the data from the 8-day untreated control group.

It is important to state that the individual mouse infection is more variable than that seen in cotton rat or hamster. The liver data are more uniform than the spleen data, but still show more variability of response than comparable data for the hamster. Nevertheless, using such large groups of animals for a trial (10 in a group), dependable observations may be made. Thus, the liver data in Table 4 show a clear dosage-response effect.

### New compounds tested.

Of the compounds tested only amphotericin B showed any appreciable amount of activity. In the first experiment this compound was active both at 300 mg/kg and 60 mg/kg, showing significantly fewer parasites than the untreated control group. However, the parasites were not eliminated from either liver or spleen, indeed some increase in numbers occurred from the 1-hour count. In this particular experiment amphotericin had about the same effect as 94 mg/kg of pentostam. The data are shown in Table 5. Amphotericin B, upon intraperitoneal injection in these hamsters in both doses, was quite toxic and produced peritonitis with adhesions. In two other experiments a more soluble form of amphotericin B, called fungizone, was shown to be active even at doses as low as 1.25 mg/kg (Table 5).

Table 5 — *The effect of amphotericin B on* L. donovani *infections in the hamster. Six animals per group*

| Treatment group | Experiment I | | Experiment II | Experiment III |
|---|---|---|---|---|
| | Group mean Total Lds in liver | *p* values | Group mean Total Lds in liver | Group mean Total Lds in liver |
| None — 1 hr. | 48 | — | 12 | 31 |
| None — 8-days | 2,445 | — | 533 | 754 |
| Amphotericin — 60 mg/kg. | 277 | $<0.001$ | — | — |
| Amphotericin — 300 mg/kg | 393 | $<0.001$ | — | — |
| Pentostam — 94 mg/kg | 327 | $<0.001$ | 8 | — |
| Fungizone — 20 mg/kg | — | — | 3 | — |
| Fungizone — 5 mg/kg | — | — | $<3$ | — |
| Fungizone — 1.25 mg/kg | — | — | — | 74 |
| Fungizone — 0.67 mg/kg | — | — | — | 277 |

Fumagillin at 2 mg/kg showed no activity, but a significant effect was noted at 20 mg/kg, a level which produced toxic manifestations in the hamsters. This observation needs further study. The inactive compounds were tested only at the doses indicated: M Sb, 60 mg/kg and 300 mg/kg; Mel B, 1.5 mg/kg an 8.0 mg/kg; tritheon, 5 mg/kg and 50 mg/kg. It is interesting to note that Fulton *et al.* (8) also found M Sb to be inactive in the hamster and cotton rat by their method. The other compounds have not been tested previously so far, as is known to the author.

## SUMMARY

An 8-day method of screening compounds for possible effect on the course of visceral leishmaniasis has continued to prove useful not only when evaluated in the hamster, but also in the cotton rat, chinchilla and mouse.

An alternative 14-day method is available for compounds suspected of more delayed action.

Amphotericin B, an antifungal agent, is shown to inhibit the development of the parasites in the hamster, but not to eliminate them.

## BIBLIOGRAPHY

1. Franchino, E. M., Grun, J., and Stauber, L. A. — «A method for screening compounds against visceral leishmaniasis in the hamster», *J. Parasitol.*, **42** (4, Sect. 2): 11, 1956.
2. Stauber, L. A., Franchino, E. M., and Grun, J. — «An eight-day method for screening compounds against visceral leishmaniasis in the hamster», *J. Protozool.*, **5**: 269-273, 1958.
3. Stauber, L. A. — «Leishmaniasis in the hamster», in W. H. Cole, ed. *Some Physiological Aspects and Consequences of Parasitism* (New Brunswick, Rutgers U. Press, 1955): 76-90, 1955.
4. Grun, J., and Stauber, L. A. — «A comparative study of experimental leishmaniasis in the mouse, Mongolian gerbil, hamster, white rat, cotton rat and chinchilla», *J. Parasitol.*, **43** (5, Sect. 2): 16-17, 1957.
5. Stauber, L. A. — «Host resistance to the Khartoum strain of *Leishmania donovani*», in *Symposium on Resistance and Immunity in Parasitic Infections*. Rice Inst. Pamphlet Ser **45** (1): 80-96, 1958.
6. Goodwin, L. G. — «The chemotherapy of experimental leishmaniasis. I. The spleen as an index of infection in the Syrian hamster», *Trans. Roy. Soc. Trop. Med. & Hyg.*, **44**: 717-728, 1944.

7. Goble, F. — Personal communication.
8. Fulton, J. D., Joyner, L. P., and Chandler, R. L. — «Studies on protozoa. II. The golden hamster *(Cricetus auratus)* and cotton rat *(Sigmodon hispidus)* as experimental hosts for *Leishmania donovani*», *Trans. Roy Soc. Trop. Med. and Hyg.*, **44**: 105-112, 1950.

# DE LA LEISHMANIOSE VISCÉRALE EN U.R.S.S.

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De la grande quantité de matériels littéraires et de données de recherche dont nous disposons on peut voir que les particularités du caractère de l'ambiance déterminent l'originalité de la diffusion et de l'épidémiologie de la leishmaniose viscérale dans de différents pays. La situation géographique et le relief du terrain avec ses éléments climatiques généraux (température de l'air, humidité relative et absolue, quantité des précipitations atmosfériques, vents), la durée des saisons, ainsi que les conditions de la vie quotidienne créent pour les localités déterminées les différences épidémiologiques de l'accomodation des espèces de micro-organismes — agents morbifiques et leurs transporteurs phlébotomes.

En même temps, à cause de l'établissement de certains rapports entre l'organisme humain et le parasite (leishmans) dans les conditions concrètes de l'ambiance, se sont déterminées les différentes variantes de l'écoulement clinique de la maladie. Il n'y a pas de doute que la pathogénie de la leishmaniose viscérale doit être caractérisée par certaines particularités de foyer.

À la suite de tout cela se sont formés les foyers particuliers de la leishmaniose viscérale. Cette maladie est largement répendue dans le globe — régions tropicales et subtropicales —, mais ses aréas sont entrecoupés. Dans les foyers différents les transporteurs des agents morbifiques sont les différentes espèces de phlébotomes; les parasitifères de la composition de la faune domestique et sauvage du foyer, eux aussi, sont différents.

Il est bien possible qu'il existe plus de variantes géographiques de la leishmaniose viscérale que nous les connaissons (V. M. Jdanov,

1953). Dans la littérature qui nous est accessible il n'y a pas de renseignements suffisants concernant les caractéristiques biologiques du foyer des leishmans, les particularités épidémiologiques, ainsi qu'il n'y a pas de données plus ou moins complètes relatives à l'entrecoupement naturel de cette maladie.

L'une des différences de l'épidémiologie des affections est celle, par exemple, qui s'est révélée en Inde sous la forme isolée: kala-azar. L'agent morbifique du kala-azar est *L. donovani* (Laveran et Mesnil, 1903). On peut considérer le kala-azar comme une pure anthroponose.

Le kala-azar indien rappelle, par une série de ses indices épidémiologiques et cliniques, la leishmaniose viscérale du Soudan, dont l'agent morbifique est considéré *L. donovani* var. *archibaldi* (Archibald et Mansour, 1936). La leishmaniose viscérale qui se rencontre au Kenya ressemble, elle aussi, au kala-azar, bien que dans l'épidémiologie de cette affection se soient faites des modifications sensibles. R. B. Heisch (1957) a trouvé dans la région de Marigate, où a été enregistrée la rechute de la leishmaniose viscérale parmi les êtres humains, les leishmans chez les écureuils zébrés *(Xerus rutilus)* qui étaient pathogènes pour le hamster. Les leishmans y ont été trouvés aussi chez les lézards, mais ils n'étaient pas pathogènes pour les hamsters. Ces faits démontrent qu'au Kenya la leishmaniose viscérale appartient au nombre des zoonoses avec l'entrecoupement naturel.

D'autre part, la leishmaniose viscérale méditerranéenne se caractérise par d'autres particularités par rapport au kala-azar indien. La maladie se rencontre plus spécialement dans les endroits relativement moins humides. Autrefois elle se manifestait presque exclusivement parmi les enfants âgés jusqu'à 5 ans et, par conséquent, elle a été nomé par Ch. Nicolle «la leishmaniose infantile», don l'agent morbifique est *L. infantim* (Ch. Nicolle, 1903). À présent cette maladie se révèle de plus en plus chez les adultes (Espagne, Italie méridionale, Grèce).

*P. perniciosus, P. major* et *P. papatasii* sont considérés comme les transporteurs de l'agent morbifique de la leishmaniose viscérale dans les régions méditerranéennes. C'est le chien qui y est le parasitifère naturel des leishmans. La leishmanioïde épidermique n'est pas remarquée. Dans le sang périférique des malades on trouve rarement les leishmans. La résistance de l'agent morbifique contre les préparations de la stibine est particulièrement marquée. La maladie de

Borovsky (la leishmaniose épidermique) se rencontre rarement dans les régions méditerranéennes.

Il y a la preuve de considérer le kala-azar épidémique de l'Inde et du Soudan et la leishmaniose viscérale épidémique des régions méditerranéennes comme les variétés de la leishmaniose viscérale (N. Latychev et T. Pavalichina, 1950). L'aréa de celle-ci englobe un grand territoire en commençant de la péninsule de Kouantoun jusqu'à la Péninsule Ibérique et passe à travers l'océan Atlantique dans les pays de l'Amérique Latine.

Toutefois, nous nous convaincons que dans la diffusion de la leishmaniose viscérale méditerranéenne, ainsi que dans les détails de ses particularités épidémiologiques et l'écoulement clinique sont remarquées les différences foyères dépendant de l'endroit où cette maladie se rencontre. Il est probable que dans les régions méditerranéennes même existent les différentes variantes de la leishmaniose viscérale méditerranéenne, ce qui nécessite de recherches spéciales.

Les variantes de Chine et d'Asie Centrale de la leishmaniose viscérale méditerranéenne se caractérisent par les déviations foyères du tableau épidémiologique et clinique. Dans ces régions la maladie frappe la population adulte. Il nous paraît que le nom de l'agent morbifique *L. infantum* n'exprime plus toute la substance de cette maladie et, par conséquent, il est plus rationnel d'appeler cet agent morbifique *L. donovani* var. *mediterranea*. Il n'y a pas de doute que cette variante de l'agent morbifique doit avoir encore plusieurs variantes.

En U.R.S.S. la leishmaniose viscérale se rencontre dans les républiques de l'Asie Centrale (R.S.S. d'Ouzbékie, R.S.S. de Tadjikie, R.S.S. de Tourkménie, R.S.S. de Kirghizie et Sud de R.S.S. de Kazaqui) et de Transcaucasie (R.S.S. d'Azerbaïdjan, R.S.S. de Géorgie et R.S.S. d'Arménie).

La leishmaniose viscérale d'Asie Centrale se distingue par ses particularités épidémiologiques et cliniques des autres variantes géographiques foyères de cette maladie et dans la zone de sa diffusion se rencontrent les déviations à l'intérieur du foyer. Ici la maladie se rencontre dans les villes. Ce sont les chiens qui sont considérés comme les parasitifères naturels essentiels des leishmans. Quelques foyers de Tadjikistan font l'exception, par exemple le bassin du fleuve de Vakhch, où les enfants et les adultes sont également frappés par cette

maladie et les parasitifères naturels des leishmans, d'après les données de N. Latychev, de A. Krioukova, de T. Povalichina et de V. Tchernychev (1947), sont les chacals *(Thos aureus)*. Ainsi, dans la R.S.S. de Tadjikie la leishmaniose viscérale se caractérise par l'entrecoupement naturel, comme la maladie de Borovsky du type villageois.

Nous trouvons des particularités dans la diffusion et dans l'épidémiologie des leishmanioses en Géorgie qui les distinguent non seulement du kala-azar indien et soudanien, mais aussi de ceux de la Chine, du Méditerranée et de l'Asie Centrale, rappelant par leur sporadicité la variante du foyer le plus nordique du monde de cette maladie — Sud de R.S.S. de Kazaqui (45° latitude nord), ainsi que celle d'Amérique du Sud, dont l'agent morbifique est considéré *L. donovani* var. *chagasi* (da Cunha et Chagas, 1937).

Les foyers de Géorgie se caractérisent par des extinctions périodiques, des réapparitions et des déplacements. La leishmaniose viscérale, comme la maladie de Borovsky, s'y distingue par une stricte sporadicité. Les affections apparaissent soudainement sous la forme de cas isolés indépendamment de la maison où a été enregistré dans le passé ou à présent le malade. Cette maladie se rencontre dans les villes et surtout dans les villages. Les transporteurs de l'agent morbifique de la leishmaniose viscérale sont *P. chinensis* et *P. kandelakii*, qui se trouvent dans les trous des animaux sauvages. En U.R.S.S. la limite verticale de la diffusion de la leishmaniose viscérale atteint 970 m au-dessus du niveau de la mer (Erevan, R.S.S. d'Arménie), 1.020 m (Och, R.S.S. de Kirghizie), 1.240 m (Dmanisi, R.S.S. de Géorgie).

En Géorgie la leishmaniose viscérale frappe surtout les enfants; souvent elle se manifeste aussi chez les adultes. Le tableau de son écoulement clinique se distingue de celui des autres variantes de la maladie. Les leishmans dans le sang périférique se révèlent bien moins rarement que dans les cas de kala-azar indien. Dans la peau saine des malades les leishmans ne se trouvent pas; l'affecté primaire est semblable à l'affecté des cas des variantes d'Asie Centrale et de Soudan de la leishmaniose viscérale. La peau du visage prend, à côté de la pâleur, un teint terreux. La courbe de température est d'un type ondulant comme en cas de brucellose, mais toutefois dans les cas isolés on remarque des rémissions sans température d'une durée inégale. L'agent morbifique résiste bien contre les préparations de la

stibine; c'est pourquoi le traitement exige une grande quantité de préparations et dure plus longtemps qu'en Asie Centrale et surtout qu'en cas du traitement de kala-azar indien. La leishmanoïde épidermique — aggravation postérieure de kala-azar —, ne se rencontre pas. La leishmaniose viscérale qui se rencontre en Géorgie (Géorgie orientale exclue) a beaucoup de traits communs avec celle des autres républiques de Transcaucasie et surtout de la R.S.S. d'Azerbaïdjan, plus spécialement par sa sporadicité de diffusion, se qui doit être lié à l'entrecoupement probable naturel. Comme la leishmaniose viscérale de Transcaucasie se distingue, d'une certaine manière, des autres variantes de cette maladie, il serait plus rationnel d'appeler son agent morbifique *L. donovani* var. *transcaucasica*.

Les agents morbifiques cités ci-dessus sont les variétés ou les variantes de l'espèce essentielle de l'agent morbifique de la leishmaniose viscérale qui se sont formées à cause de leur accomodation à l'ambiance des terrains géographiques ou des régions. Il n'y a pas de doute que l'existence de différentes variantes de la leishmaniose viscérale est aussi liée à la présence de leurs foyers naturels.

# NATURAL NIDI OF SKIN AND VISCERAL LEISHMANIASIS

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Twenty years have elapsed since the theory of natural nidi of certain diseases of man was formulated (E. N. Pavlovsky, 1938).

The theory of natural nidi may be briefly summarized as follows: a natural nidus of certain disease of man is a section of territory with definite geographic landscape, the biotopes of which form a definite biocenosis. In this territory, the etiological agents of one or more infectious diseases acting as a participant circulate from one animal to another through the vectors, i.e., the blood-sucking arthropods.

This interrelationship between the organisms within the natural nidus had developed in the course of evolution without the participation of human beings. When susceptible persons enter the natural nidus, they may be attacked by the infective arthropods and contract the disease. Thus, zoonosis becomes arthropo-zoonosis.

Exploratory materials gathered by numerous expeditions from the remotest rural districts never before known to man have proven the existence of natural nidi of skin and visceral leishmaniasis.

Summarizing our investigations, we ascertained a definite relation between natural nidi of skin and visceral leishmaniasis and geographic landscapes of distinct character. Semi-deserts and deserts bear skin and visceral leishmaniasis nidi.

In the territories with different landscapes, the natural nidi of the diseases are localized in small limited areas which are technically known as elementary nidi (E. N. Pavlovsky) or micronidi (P. A.

Petrishcheva). Many times such micronidi may be a hole or a colony of holes of rodents and other wild mammals. For instance, the hole of porcupine (*Hystrix hirsutirostris saturnini* Müller) may be the nidus of skin and visceral leishmaniasis as well as tick borne relapsing fever; likewise, the hole of the jackal (*Canis aureus aureus* Linn.) may be the nidus of visceral leishmaniasis. Similarly, the colony of holes of large sand rat (*Rhombomys opimus* Licht.) may serve as the natural nidus of skin leishmaniasis, tick borne rechettsiosis and Q fever; and that of spermophile (*Spermophilopsis leptodactylus* Liont) as the nidi of skin leishmaniasis and tick borne relapsing fever. Groups of holes of hedgehog and Indian rat may also serve as the nidi of skin leishmaniasis. Therefore, the above mentioned mamals should be considered as real reservoirs of these diseases. The vectors disease agents: *Ph. papatasii, Ph. sergenti, Ph. caucasicus, Ph. mongolensis, Ph. chinensis, Ph. arpaklensis.*

Mountainous regions with moderate or warm climate are of great epidemiological interest. In Central Asia, on the mountain and in its vicinity with an altitude up to 1200-1500 m above sea level, many kinds of shelters of wild animals can be found, namely: caves, grottoes, deep clefts and gaps, each with its own microclimate.

It is the multiformity of the living conditions in the limited areas of the mountain and its vicinity that ensures the existence of natural nidi of various diseases. For example, along the coast of upper Sumbara River (Kopet-Dahg, Turkmenia) natural nidi of skin and visceral leishmaniasis, tick borne spirochetosis and Q fever are found. However, not all the natural nidi give rise to similar morbidity rates in the mountainous as well as in the flat areas. Skin leishmaniasis in the desert of Karn-Kum may give rise to very intensive epizootics and cause infection of the new comers. This occurs when there are few vectors and susceptible animals in the natural nidi of skin leishmaniasis. On the contrary, in case the density and the number of species of phlebotomus and wild animals are great in the above mentioned areas, epizootics of skin leishmaniasis would proceed sluggishly, rarely infecting man. These phenomena may be ascribed to the presence of natural «blind alley», where leishmania organisms find themselves in association with many species of animals and vectors. The term «blind alley» means that species of animals and arthropods in which the disease agents happen to be, and in which they either

perish quietly or get no chance of invading another host organism (E. N. Pavlovsky).

Study of regional pathology in the light of landscape epidemiology is of great practical value. Once the natural connection between various diseases and the territories of different landscapes has been established, the landscape itself may become the index of its risk to infect newcomers. This would enable us as early as possible to provide and carry out the necessary general prophylactic measures.

The elucidation of biological and other factors of the existence of the natural nidi of diseases enables us not only to carry out prophylactic measures, but also to eradicate these nidi in territories of important economical development.

# AROMATIC DIAMIDINES [1]

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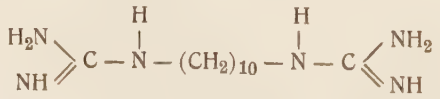
The direct trypanocidal action of synthalin (decamethylene diguanidine) was first demonstrated by Lourie and Yorke (1937). Jansco and Jansco (1935) had previously noted that this compound exerted a curative effect when injected into mice infected with pathogenic trypanosomes, but by a process of false deduction and incomplete experimentation they attributed this effect to the hypoglycaemia producing properties of the drug rather than to any direct action on the trypanosomes. The discovery of the direct trypanocidal activity of synthalin was of great academic interest and opened a wide new field of chemotherapeutic research in view of the fact that the chemical constitution of synthalin is essentially different from that of all hitherto known trypanocidal substances, consisting of two guanidine groups connected by a long alkylene chain, thus:



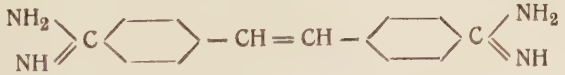
## TRYPANOCIDAL ACTIVITY

King, Lourie and Yorke (1937) prepared and examined a large number of compounds related to synthalin: guanidines, isothioureas amidines and amines with alkyl and alkylene chains. It was found that certain of the diamidines exhibited a powerful trypanocidal action

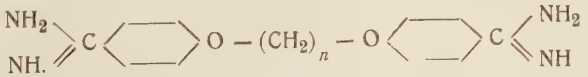
[1] The paper was presented *in absentia*.

*in vitro* and *in vivo*, the most active being *n*.undecane-1:11-diamidine. The structural feature common to all the active compounds appeared to be the possession of an inert central carbon chain with terminal polar groups of strongly basic nature. King, Lourie and Yorke (1937, 1938) observed progressive increase in trypanocidal activity with increasing length of the central carbon chain until $(CH_2)_{10}$-$(CH_2)_{16}$. Increase in the alkyl chain beyond $(CH_2)_{16}$ was associated with a decrease in trypanocidal activity. They considered (Yorke, 1939) that trypanocidal activity depended on the biologically active polar groups combined with a certain molecular size which was provided by the central inert carbon chain and suggested that the replacement of this chain by an inert aromatic structure giving approximately the same molecular weight might produce some interesting compounds.

The investigation was extended by Dr. A. J. Ewins and the research staff of May & Baker to a series of aromatic compounds containing the amidine group. A large number of derivatives was prepared by varying the following (Ashley *et al.*, 1942): (*a*) length of the alkane chain linking the amidino phenyl residues, (*b*) substitution and variation of the divalent linkage, (*c*) change in position of the amidine groups and (*d*) introduction of -O-, -N-, and -S- linkages in the alkane chain. Preliminary investigation showed that a number of these compounds exhibited high trypanocidal activity *in vivo*, single injections of a small fraction of the tolerated doses sufficing to cure infected animals. Wider investigation of these compounds was undertaken by Lourie and Yorke (1939), who found the most active compounds to be 4:4′-diamidino stilbene (later known as stilbamidine, or M & B 744):



and members of the series:



particularly 4:4′-diamidino diphenoxy propane, where $n=3$ (later known as propamidine or M & B 782) and 4:4′-diamidino diphenoxy

pentane, where $n=5$ (later known as pentamidine or M & B 800). It was found in this series that the trypanocidal activity increased with the length of the alkane chain up to a point where $n=5$; beyond this point the compounds fall off in activity, until when $n=10$ they are comparatively inert. Minor changes in the carrier chain such as the introduction of one or two methyl groups did not alter the potency of the compounds, provided the integrity of the polar aromatic diamidine group was preserved. Any change in this group, however, completely destroyed trypanocidal activity. The therapeutic activity of the most active compounds compared favourably with that of the various aromatic arsenicals previously used in the treatment of trypanosomiasis.

## ACTION IN LEISHMANIASIS

Having demonstrated the therapeutic activity of the diamidines in experimental trypanosomiasis, Warrington Yorke sent a quantity of 4:4′diamidino stilbene to Prof. Saul Adler in Jerusalem with the request that he would test its effects in hamsters infected with visceral leishmaniasis. Adler did so and found (Adler and Tchernomoretz, 1942) that the drug had a marked therapeutic effect in this infection. This was of great interest as it was the first instance of a drug not containing antimony with a therapeutic action on leishmania infection. In view of these results, Adams and Yorke (1939) administered 4:4′-diamidino stilbene to a severe case of Indian kala-azar and obtained an apparent cure after only eight daily injections of 1 mg per kilo body weight. Warrington Yorke then wrote to me at Khartoum asking if I could arrange trials in Sudan kala-azar, which is a particularly virulent type of the disease (Kirk, 1947). My colleagues and I were very pleased to do so and obtained remarkable immediate results with a much higher cure rate than any antimony preparation had given in Sudan kala-azar (Kirk and Sati, 1940*a*, 1940*b*) using stilbamidine, pentamidine and propamidine. Good results with stilbamidine were also reported by Adler and Rachmilewitz (1939) from Jerusalem and by Napier and Sen (1940) from Calcutta. In 1942 Napier, Sen Gupta and Sen published a detailed account of 101 cases of Indian kala-azar treated with stilbamidine, with an inmmediate

cure rate of 98 %, slightly better than the best results previously obtained with antimony in Indian kala-azar.

## INSTABILITY OF STILBAMIDINE

With Italy's entry into the war in 1940 the possibility of prolonged campaigns in the kala-azar areas of the Sudan and East Africa had to be considered. After many anxious months at the beginning of the war the fear of invasion passed from the Sudan and sufficient allied forces had been accumulated there for offensive action, the invasion of Italian East Africa. It was realised that this campaign would have to be fought across the almost uninhabited no man's land on the Sudan Abyssinian border, which was known to be heavily infected with kala-azar. In view of the results reported above, stocks of stilbamidine were obtained in preparation for a heavy incidence of this disease.

The incidence of kala-azar in the East African campaigns was considerable and stilbamidine was used for treatment. Immediate results of treatment similar to those previously reported were at first obtained. But, as time went on, reports of various toxic effects became frequent, the most distressing being a series of symptoms suggesting hepato-renal failure and ending fatally. Investigation revealed that cases in which these toxic effects occurred had been treated with ready made up solution, whereas in the early cases the solution had been freshly made from the powder immediately before each injection (Kirk and Sati, 1943). Toxicity tests in mice with stock and freshly prepared solution showed the former to be much more toxic than the latter (Kirk & Henry, 1944). The results were communicated to Prof. Warrington Yorke, who was able to show that the factor responsible for the increased toxicity was ultra-violet light; that the increased toxicity was accompanied by loss of trypanocidal activity and disappearance of the chemical properties attributable to the ethylenic linkage; and that these changes occurred only in the unsaturated compounds, saturated compounds such as pentamidine and propamidine being unaltered by exposure to light (Fulton and Yorke, 1942).

The chemistry of the changes which stilbamidine can undergo in solution is of great interest and complexity and has been described

by Henry (1943, 1945, 1946, 1948). The drug as prepared by the makers is *trans*-4:4′-diamidino stilbene and on irradiation aqueous solutions undergo two concurrent changes, dimerisation and *cis-trans* isomerism, the proportion in which the products of the two reactions are formed depending on concentration and temperature. These changes do not take place in the dark, but in sunlight at all concentrations and temperatures they proceed until equilibrium is established between *cis*-stilbamidine and *trans*-stilbamidine, the equilibrium thereafter slowly drifting owing to dimerisation of the *trans* form to produce 1, 2, 3, 4-tetra (4′-amidino phenyl) cyclobutane, with disappearance of the ethylenic linkage. It is the formation of this compound which is mainly if not entirely responsible for the increased toxicity of solutions exposed to light (Kirk and Henry, 1944). The *cis-trans* change is freely reversible; the dimerisation of *trans*-stilbamidine is practically irreversible in sunlight, while *cis*-stilbamidine has no tendency to dimerise except indirectly through the *trans* form. Henry established also that the amidine groups are not indefinitely stable, but undergo slow hydrolysis with conversion into amido groups and liberation of ammonium chloride. This reaction occurs in the dark and appears to depend primarily on temperature and pH of the solution. The first hydrolysis product, *trans*-4-amido-4′-amidino stilbene hydrochloride, was shown to be more toxic for mice than the parent stilbamidine hydrochloride (Kirk and Henry, 1944). Its formation in the body — for which conditions of pH and temperature would be favourable — from stilbamidine absorbed and retained for long periods may possibly account for some of the delayed toxic effects which have been observed (Henry, 1948).

## CLINICAL TOXICITY

Since the photochemical instability of aqueous solutions of stilbamidine was established the drug has been supplied as a powder in which no deterioration has been observed to occur after months or years of storage in the dark, and freshly prepared solutions of stilbamidine have given no evidence of hepatic or renal injury. However both immediate and late toxic reactions have been observed to follow the administration of the diamidines and these have been

sufficiently important to influence the choice of drug in the treatment of kala-azar and trypanosomiasis.

*Immediate reactions.* — During or immediately after the intravenous injection the following reactions have been observed; fall in blood pressure, tachycardia, vertigo, salivation, sweating, headache, nausea, vomiting, dyspnoea, syncope, formication, faecal and urinary incontinence, lethargy, oedema of the face and eyelids, epileptiform attacks. Most of these reactions are due to the immediate fall in blood pressure following intravenous injection of the drugs, are usually transient in nature and pass off in a few moments. In some patients a modified Herxheimer reaction occurred after injections of the diamidines as long as the leishmania infection was still active (Kirk and Satti, 1940*b*). In others thrombophlebitis at the site of intravenous injection has sometimes been troublesome.

*Late toxic reactions.* — The commonest late toxic effect of stilbamidine, first described by Napier and Sen Gupta (1942), is a peculiar neuropathy characterised by subjective disturbances and dissociated anaesthesia, most commonly involving the trigeminal nerve. The neuropathy usually develops some months after stilbamidine treatment, and is rather difficult to explain. Napier and Sen Gupta (1942) concluded that the lesion is a central one affecting the chief sensory nuclei in the pons. Sen Gupta (1943) suggested that the selective affinity of the drug for the trigeminal nucleus is probably due to the ethylenic compound as in trichlorethylene, which has a similar selective toxic action on the trigeminal nucleus. Subsequently, Collard and Hargreaves (1947) also expressed the same opinion. The fact that complete recovery has been observed in some cases (Sati, 1949) is difficult to reconcile with a central nuclear degeneration, since with such a lesion one might expect the sensory loss to be permanent. Oastler and Fidler (1946) administered stilbamidine to dogs and found very definite lesions in the central nervous system — cortex, cerebellum, thalamus, caudate nucleus, brain stem and spinal cord — but no localization in the pons. However, it is doubtful if these findings can be used to interpret toxic effects in man, since dogs are more susceptible than other animals to the toxic effects of stilbamidine (Lourie and Yorke, 1939). In cases reported by Sati (1949) in the Sudan the neuropathy was not restricted to the fifth nerve, but involved also the seventh and ninth, cranial

nerves and, in some cases, the cervical and dorsal spinal nerves as well. This is in aggreement with the findings of Collard and Hargreaves (1947) in British service-men, among whom a high proportion showed neuropathy extending to the waist.

The fact that neuropathy develops some months after the end of treatment is of interest and suggests that it may be related to chemical changes in stilbamidine retained in the body. Sati (1949) has shown that many cases continue to excrete stilbamidine in the urine for several years after treatment is stopped. He records the case of one patient who was treated with an old, exposed solution in which considerable photochemical changes had taken place and who developed the neuropathy while he was still having the injections, i.e., without the usual latent period after the end of treatment. Henry (1948) has suggested that conditions of pH and temperature in the body would be favourable for the formation of trans-4.amido-4-amidino stilbene from stilbamidine absorbed and retained for long periods, and this may possibly account for some of the delayed toxic effects. Sati (1949) has pointed out that in Sudanese patients the signs and symptoms of the neuropathy tend to occur on the sites of the body exposed to the action of sunlight by the local fashion dress, i.e., the face, neck and chest. Snapper (1947), who had studied the effects of stilbamidine in multiple myelomatosis, recommends protecting the patients from sunlight during and after treatment, lest some stilbamidine remaining in the skin may be altered by it and cause selective nerve injury.

## AROMATIC DIAMIDINES AND LEISHMANIASIS: PRESENT POSITION

In addition to stilbamidine, propamidine and pentamidine, the following two aromatic diamidines have been used in the treatment of kala-azar: 4.4′-diamidino diphenyl ether (also known as phenamidine, or M & B 736) and 4:4′-diamidino-2-hydroxy stilbene (also known as hydroxystilbamidine). From a review of reports on the chemotherapy of kala-azar with aromatic diamidines it appears that stilbamidine is the most powerful leishmanicidal agent in the series with hydroxystilbamidine a close second. Pentamidine is relatively less effective and phenamidine even less so (Sen Gupta, 1953). Pro-

pamidine has proved very toxic and for this reason has not been used to any great extent in the treatment of kala-azar. The occurrence of post stilbamidine neuropathy and other toxic effects has restricted the use of stilbamidine, with the result that pentamidine has become the most commonly used of the diamidines. In the writer's opinion, hydroxystilbamidine may be worth more extensive trials than it has had. Most ordinary cases of kala-azar will probably continue to be treated successfully with the pentavalent antimonials, urea stilbamine, neostibosan, pentostam and protostib, aromatic diamidines being reserved for antimony resistant cases, cases complicated by pulmonary tuberculosis and antimony sensitive cases. The apparent development of diamidine resistant strains of leishmania has been observed by Schoenbach and Greenspan (1948), but the practical importance of this is still uncertain.

The aromatic diamidines appear to be no more efficacious than antimonials in post kala-azar dermal leishmaniasis. They have not been used to any great extent in the treatment of oriental sore or American leishmaniasis. The curious form of oro-pharyngeal leishmaniasis seen in the Sudan was found by Humphreys (1942) to respond to pentamidine, however, and Correa and Brito (1954) report the successful treatment of 15 severe cases of South American mucosal leishmaniasis with diamidino diphenoxy pentane (lomidine). All cases were apparently cured and four of them seen after three to four months showed no evidence of relapse.

## GENERAL CHEMOTHERAPEUTIC ACTIVITY OF AROMATIC DIAMIDINES

In addition to their action on trypanosome and leishmania infections the aromatic diamidines have been shown to have a wide range of chemotherapeutic activity (Schoenbach and Greenspan, 1948; Kirk, 1957). The growth of many bacteria, protozoa and fungi is inhibited by them in concentrations far below those found toxic to experimental animals and human beings. The antibacterial properties of propamidine against gram positive cocci compare favourably with those of the sulphonamide drugs and penicillin, as does also its therapeutic effect in clostridial infections in mice. It is comparatively

free from toxic effects when used by local application and was found clinically to compare favourably with sulphathiazole and penicillin in the treatment of burns and purulent open war wounds.

Among fungal infections stilbamidine has been found valuable in the treatment of actinomycosis, sporotrichosis, granuloma paracoccidioides and North American blastomycosis due to *Blastomyces dermatidis* (Kirk and Morgan, 1958), being a specific remedy for the last condition.

Stilbamidine was found to produce dramatic relief of the severe generalized bone pains of multiple myelomatosis, and there is evidence that the diamidines, particularly stilbamidine, are able to inhibit the multiplication of tumour cells, probably by interfering with nucleoprotein metabolism. The diamidines and other synthetic antiseptics have been somewhat overlooked since the advent of penicillin and other antibiotics, but they are still worth further investigation. The property of displacing basic proteins from combination with nucleic acid makes stilbamidine a compound of unique interest in the search for new chemotherapeutic substances.

## BIBLIOGRAPHY


Adams, A. R. D., & York, W. — *Ann. trop. Med. & Parasit.*, **33**: 323, 1939.

Adler, S., & Rachmilewitz, M. — *Ibid.*, **33**: 327, 1939.

Adler, S., & Tchernomoretz, I. — *Ibid.*, **35**: 9, 1941.

Ashley, J. N., Barber, H. J., Ewins, A. J., Newberg, G., and Self, A. D. H. — *J. Chem. Soc.*, Part I, **20**: 103, 1942.

Collard, P. J., & Hargreaves, W. H. — *Lancet*, **2**: 686, 1947.

Correa, A., & Brito, G. da R. — *Bull. Soc. Path. exot.*, **47**: 513, 1954.

Fulton, J. D., & Yorke, W. — *Ann. trop. Med. & Parasit.*, **36**: 134, 1943.

Henry, A. J. — *Nature*, **152**: 692, 1943.

Henry, A. J. — *J. Chem. Soc.*, Part II, 870, 1945.

Henry, A. J. — *Ibid.*, Part II, 1156, 1946.

Henry, A. J. — *Brit. J. Pharmacol. & Chemotherapy*, **3**: 163, 1948.

Hunphreys, R. M. — *Ann. trop. Med. & Parasit.*, **36**: 9, 1942.

Jansco, H. von, & Jansco, H. von — *Ztschr. f. Immunitatsforsch. u. exper. Therap.*, **86**: 1, 1935.

King, H., Lourie, E. M., & Yorke, W. — *Lancet*, **2**: 1360, 1937.

King, H., Lourie, E. M., & Yorke, W. — *Ann trop. Med. & Parasit.*, **32**: 177, 1938.

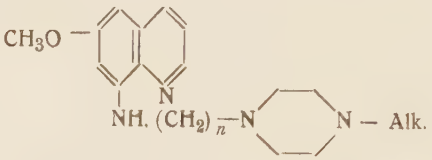
Kirk, R. — *Trans. R. Soc. trop. Med. & Hyg.*, **40**: 459, 1947.

KIRK, R. — *Proc. Alumni Assoc. Malaya,* **10**: 14, 1957.
KIRK, R., & HENRY, A. J. — *Ann. trop. Med. & Parasit.,* **38**: 99, 1944.
KIRK, R., & MORGAN, H. V. — In the press. 1958.
KIRK R., & SATI, M. H. — *Ann. trop. Med. & Parasit.,* **34**: 83, 1940*a*.
KIRK, R., & SATI, M. H. — *Ibid.,* **34**: 131, 1940*b*.
KIRK, R., & SATI, M. H. — *Ibid.,* **37**: 34, 1943.
LOURIE, E. M., & YORKE, W. — *Ibid.,* **31**: 435, 1937.
LOURIE, E. M., & YORKE, W. — *Ibid.,* **33**: 289, 1939.
NAPIER, L. E., & SEN, G. N. — *Ind. med. Gaz,* **73**: 720, 1940.
NAPIER, L. E., & SEN GUPTA, P. C. — *Ibid.,* **77**: 71, 1942.
NAPIER, L. E., SEN GUPTA, P. C., & SEN, G. N. — *Ibid.,* **77**: 321, 1942.
OASTLER, E. G., & FIDLER, H. K. — *Trans. R. Soc. trop. Med. y Hyg.,* **39**: 533, 1946.
SATI, M. H. — *Ann. trop. Med. & Parasit.,* **43**: 4, 1949.
SCHOENBACH, E. B., & GREENSPAN, E. M. — *Medicine,* **27**: 327, 1948.
SEN GUPTA, P. C. — *Ind. med. Gaz.,* **78**: 537, 1943.
SEN GUPTA, P. C. — *Ibid.,* **88**: 20, 1953.
SNAPPER, I. — *J. Amer. med. Assoc.,* **133**: 157, 1947.
YORKE, W. — Personal communications. 1939.

Dr. L. Goodwin (U.K) — I have tried Dr. Stauber's method and it seems to work very well. Those of us who are still testing new compounds against hamster leishmaniasis should be grateful to him.

I would like to tell you of a new series of active compounds which were synthesized by Dr. L. P. Walls and tested by Miss E. Beveridge and myself in hamster kala-azar.

They are:




A series of 8-aminoquinolines with piperazine in the side-chain. The methylene chain —$(CH_2)_n$— may have 3 to 6 carbon atoms in the side-chain.

These compounds are active when given by mouth. 8-aminoquinoline derivatives have certain toxic side-effects and we shall not administer any one of these substances to man until we are certain that it is not more toxic than primaquine.

Dr. M. H. Sati (Sudan) — Those of us who have to treat kala-azar would welcome, I am sure, the new compounds just now notified — one of these is amphotericin «B» — in experimental animals.

In the Sudan, pentostam had been extremely satisfactory to start with, but with the passing of the drug into more general use there is drug resistance developed. This resistance is sometimes absolute and resort has to be made to the diamidines.

New drugs are, under the circumstances, greatly welcomed.

Dr. P. Giraud (France) — Nous avons depuis 10 ans une technique qui nous permet d'avoir de façon régulière guérison du kala-azar dans un délai de 2 à 3 mois. Nous avons traité ainsi plus de cent malades sans aucun décès et la guérison a été définitive.

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*(Continued)*

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